UNIVERSITI PUTRA MALAYSIA

GROWTH AND PHYSIOLOGICAL CHARACTERISTICS OF Melaleuca cajuputi Powell PLANTED IN CONTAMINATED BRIS SOIL

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GROWTH AND PHYSIOLOGICAL CHARACTERISTICS OF *Melaleuca cajuputi* Powell PLANTED IN CONTAMINATED BRIS SOIL

By

NOOR LIYANA ATHIRAH BT MUHAMAD

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

December 2014

DEDICATION

This thesis is dedicated to my lovely hubby Mohamad Syawal bin Ishak and beloved parents, Muhamad bin Dollah and Che Hamidah binti Che Sof and also not forgotten to my precious daughter, Noor Syalia Azzahra, and my elder sister, Noor Aainaa Shahirah and her husband and daughters, Mohd Rahul, Nur Rania Insyirah and Nur Raisya Izzah, my younger brothers and sister, Mohd Syairazi Syahir, Mohd Syazwan Syarif, and Noor Diyana Bahirah for their love, unstoppable support, prayer, and encouragement during my master's journey.

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

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Chairman : Hazandy Abdul Hamid, PhD

Faculty : Forestry

The uses ofsewage sludge as commercial fertilizer in agricultural activities nowadays may resulting the contaminants of groundwater by heavy metals. These heavy metals will flow through the porous medium into groundwater and may cause health problems to all living things. *Melaleuca cajuputi* had been tested for phytoremediation capabilities using selected heavy metal, i.e. Cu and Zn. Sewage sludge in Malaysia also contains Cu and Zn. The objectives of this study was to determine the heavy metal uptake by *Melaleuca cajuputi* via transport and leaching losses of solutes using simple lysimeter as well as to investigate the best concentration level of both Cu and Zn for growth and physiological attributes of this species. Totally 72 seedlings were planted in simple lysimeter pots using Beach Ridge Interspersed with Swales (BRIS) soil. A Completely Randomized Design (CRD) was used. Four different level, 0 ppm (control), 100 ppm, 300 ppm, and 500 ppm with nine replications for each treatment were used. After one month planting, growth height, diameter of plants, survival rate, chlorophyll contents and fluorescence, and gas exchange parameters were measured. The growth performances were calculated based on absolute growth rate (*AGR*) and relative growth rate (*RGR*). Chemical analyses also were done using AAS methods. Overall observation for physiological and growth performances in the end of study showed that 100 ppm of each Cu and Zn gave the highest and best results for all measurement parameters meanwhile 500 ppm of each Cu and Zn showed the lowest. The results of this study provided some useful information regarding on a better understanding about growth and physiological attributes of the selected plant species which had been proved as a potential plant to remediate contaminated site. Eventhough this study only used the simplest method of lysimeter, it is enough to expose a new and alternative method for planting design that can measure the uptake of heavy metal by plants via leaching losses correspond to phytoremediation purposes.

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

PERTUMBUHAN DAN CIRI-CIRI FISIOLOGI *Melaleuca cajuputi* Powell YANG TELAH DITANAM DALAM TANAH BRIS YANG TERCEMAR

Oleh

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Penggunaan enapcemar kumbahan sebagai baja komersil dalam aktiviti pertanian pada masa kini mungkin boleh menyebabkan pencemaran air bawah tanah oleh logam berat. Logam berat ini akan mengalir melalui liangtanah ke dalam air bawah tanah dan boleh menyebabkan masalah kesihatan kepada semua benda hidup. *Melaleuca cajuputi* telah diuji untuk mengumpul logam berat yang terpilih Cu dan Zn. Enapcemar kumbahan di Malaysia juga mengandungi Cu dan Zn. Objektif kajian ini adalah untuk menentukan pengambilan logam berat oleh *Melaleuca cajuputi* melalui pengangkutan dan larut lesap bahan terlarut menggunakan lysimeter ringkas serta untuk menyiasat tahap kepekatan yang terbaik daripada kedua-dua Cu dan Zn untuk pertumbuhan dan sifat-sifat fisiologi spesies ini. Sebanyak 72 anak benih ditanam di dalam pot lysimeter ringkas menggunakan tanah BRIS mengikut kaedah secara rawak sepenuhnya (CRD). Empat tahap kepekatan yang berbeza, 0 ppm (kawalan), 100 ppm, 300 ppm, dan 500 ppm dengan 9 replikasi sampel setiap satu telah diaplikasikan. Selepas satu bulan penanaman, ketinggian pokok, diameter pokok, kadar hidup, kandungan klorofil dan pendarfluor klorofil, dan parameter pertukaran gas telah diukur. Prestasi pertumbuhan dikira berdasarkan kadar pertumbuhan mutlak (AGR) dan kadar pertumbuhan relatif (RGR). Analisis kimia juga telah dilakukan dengan menggunakan kaedah AAS. Pemerhatian keseluruhan bagi keputusan persembahan fisiologi dan fizikal pokok pada akhir kajian semuanya menunjukkan bahawa 100 ppm bagi setiap Cu dan Zn memberikan keputusan tertinggi dan terbaik untuk semua parameter ukuran sementara itu 500 ppm bagi setiap Cu dan Zn adalah sebaliknya. Kajian ini telah memberikan beberapa maklumat yang berguna mengenai pemahaman yang lebih baik terhadap ciri-ciri fizikal dan fisiologi spesis tumbuhan terpilih yang telah dibuktikan sebagai tumbuhan yang berpotensi untuk memulihkan tapak tercemar. Walaupun kajian ini hanya menggunakan kaedah lysimeteryang paling ringkas, ia cukup untuk mendedahkan kaedah baru dan alternatif bagi reka bentuk penanaman yang boleh mengukur pengambilan logam berat oleh tumbuhan melalui larut lesap sesuai dengan tujuan phytoremediasi.

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Hopefully, for those who will read my master research project will find that all the information beneficial to them and give general idea of what this research is all about. Therefore, I hope that this research will be accepted and can be used as one of the references for those who wish to make a study in the related fields.

Thank You.

I certify that an Thesis Examination Committee has met on 10December 2014 to conduct the final examination of Noor Liyana Athirah Muhamad on herthesis entitled "Growth and Physiological Characteristics of*Melaleuca cajuputi*Powell Planted in Contaminated BRIS Soil" 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 students be awarded the degree of Master of Science.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometry
ADGR	Absolute Diameter Growth Rate
AHGR	Absolute Height Growth Rate
AGR	Absolute Growth Rate
Anet	Net Photosynthesis Rate
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
BRIS	Beach Ridges Interspersed with Swales (BRIS) soils
CF	Chlorophyll Fluorescence
Ci	Intercellular CO_2
Cu	Copper
$CuSO_4$	Copper Sulphate
CO_2	Carbon Dioxide
DMRT	Duncan Multiple Range Test
DOE	Department of Environment
DWS	Dry Weight of Sample
E	Transpiration Rate
EPA	Environmental Protection Agency
ET	Evapotranspiration
Fm	Maximal Fluorescence
Fo	Minimal Fluorescence
Fv	Variable Fluorescence
Fv/Fm	Photochemical Efficiency
Gs	Stomatal Conductance
FWS	Fresh Weight of Sample
H_2SO_4	Sulphuric Acid
kg	Kilogram
LiCor	Portable Photosynthesis System
mg	Miligram
mm	Milimeter
$NADPH_2$	Nicotinamide Adenine Dinucleotide Phosphate
NCSA	National Capacity Self-Assessment
NOx	Nitrogen Oxide
O_2	Oxygen
PAHs	Polynuclear Aromatic Hydrocarbons
Pb	Lead
ppm	Parts Per Millions
PS II	Photosystem II
PVC	Polyvinyl Chloride
RGR	Relative Growth Rate
SLA	Specific Leaves Area
TDW	Total Dry Weight
TFW	Total Fresh Weight
RDGR	Relative Diameter Growth Rate
RHGR	Relative Height Growth Rate
SPAD	Equipment used to collect chlorophyll content

SPSS	Statistical Package for Social Science
SO_2	Sulphur Dioxide
UPM	Universiti Putra Malaysia
VpdL	Leaf to Air Vapour Pressure Deficit
$ZnSO_4$	Zinc Sulphate

CHAPTER 1

INTRODUCTION

1.1 Background of study

Land or soil pollution nowadays seems to be one of the critical problems existing on earth. This pollution occurs when there was a penetration of harmful pesticides and insecticides which serve whatever their main purpose is, but otherwise bring about deterioration in the soil quality, thus making it contaminated and unfit for use. The deposition of atmospheric and industrial waste, mining waste, agricultural chemicals, waste from human activities and incidental accumulations, are only some of the sources of heavy metal contamination (Zubillaga et al., 2008). These heavy metals represent one of the most pressing threats to water and soil resources, as well as to the health of humans and other living things.

Agricultural activities become more and more important since the rapid industrialization of the economy in the last decade. The recent use sewage sludge as commercial fertilizers in agricultural activities may increase the contamination of groundwater by heavy metals. Sludge is a good fertilizer but may contain heavy metals that resulting environmental risks (Hossain, et. al, 2013). Inorganic or chemical fertilizers, which contain metal contaminants such as copper (Cu), zinc (Zn), arsenic (As) lead (Pb) and many more, may present numerous advantages because of their availability and easy and fast absorption by plants. However, leaching, wherein soil is depleted of its natural nutrients, is considerably more prevalent with fertilizer use.

Naturally, heavy metal occurs in all soils in minute quantities, but because of from various sources, such as sewage sludge, fertilizers, organic supplements, atmospheric deposition and urban industrial activities, this heavy metal resulted to accumulate in agricultural soils (Modaihshet al., 2004).Not all of these heavy metals are essential nutrients for plants and animals.Even though Zn and Cu are essential micronutrients for plant growth and physiological performance, high concentrations of these heavy metals can be toxic to plants and soils. The ingestion of such contaminants causes serious health problems to living things, especially human beings. The threat posed by heavy metals to living things is exacerbated by their long-term persistence in the environment (Yoon et al., 2006). In agricultural soil, high Cu contents usually result from the long-term use of Cu-containing fungicides and animal manure; Zn is present in extreme concentrations in the majority of industrial waste (Rossi et al., 2004).

Several technologies for remediating soils from heavy metal contaminants have been reported. Nevertheless, as Yoon stated (as cited in Cao et al., 2002 and Mulligan et al., 2001) many of these technologies (e.g. excavation of contaminated material either chemical or physical treatment) are very costly and do not attain long-term or aesthetic result. One of the easiest and most inexpensive ways to remove contaminants from the earth is the phytoremediation method. This method involves the engineered use of green plants to remedy, remove or render environmental contaminants harmless; it is a cost-effective, long-lasting and aesthetic approach to remediating contaminated sites (Yoon et al., 2006). There are several steps in phytoremediation which are transfer of metal from the bulk soil to the roots surfaces, uptake into the roots and translocation to the shoots (Romeiroet al., 2006).

Given the importance of removing heavy metals from contaminated land (Wong, 2003), an understanding of reactive plant transport in porous media is necessary to predict the fate of pollutants in soils and aquifers (Hu et al., 2007). An alternative technique is to measure the heavy metal uptake of plants via leaching losses. Outdoor leaching or percolation experiments, which are carried out under natural field conditions, generally refer to lysimeter experiments. The original application of lysimeter has elicited increasing attention in the last decade because of the recent rise in groundwater pollution and contamination. Lysimeters are essential tools for monitoring soil, plant and atmospheric conditions. According to Lazarovith (2006, as cited in Hillel et al., 1969 and Van Barel, 1961), a lysimeter can directly measure actual evapotranspiration rates and facilitate water, fertiliser and solute balance studies. As part of natural physiological processes, plants normally pump water, nutrients, solutes and organic matter from surrounding media. This potential can be used to remove, break down or stabilise contaminants in soil (Robinson et al., 2003).

The selected plant species in this study was *Melaleuca cajuputi*.It is locally known as *Gelam* or *Kayu Putih* and belongs to the Myrtaceae family. This species can produce essential oils that are suitable for medicinal purposes; the cajuput oil from *M. cajuputi* has been used as external treatment for headache, toothache, ear-ache, rheumatic cramps and fresh wounds (Lim et al., 2001). Ko Ko (2009, as cited in Doran et. al, 1994) indicated that the leaves are also used as flavouring in cooking and as a fragrance and freshening agent in soaps, cosmetics, detergents and perfumes. *M. cajuputi* naturally occurs in swamp forests between old raised sea beaches and mangroves (Lim et al., 2001). It has a potential to survive in sea beaches soil which both waterlogged and well-drained soils. Lots of this species occurs naturally along the seaside and riverside in Setiu, Terengganu. Therefore, all the seedlings were taken from Setiu, Terengganu and was conducted using beach ridges interspersed with swales (BRIS) soil as a planting medium to maintain natural occurence of this species.

Hence, in order to overcome the contaminated groundwater problems resulting from various causes which had been discussed above, the selected plant *Melaleuca cajuputi* was used to clean up the selected heavy metal, Cu and Zn which naturally found in sewage sludge. Besides, there were many of previous study had been discussed about the capability of *Melaleuca cajuputi* to survive in contaminated site. Moreover, the capability of this species to survive in water-logging conditions enable the isolation of liquid-form Cu and Zn that leach to groundwater systems—a task that can also be performed using a simple lysimeter method. This study anyhow was more focused on physiological performances and growth response of *Melaleuca cajuputi*which planted in BRIS soil using a simple lysimeter method. The result was expressed according to the measurements of chlorophyll content and fluorescence, gas exchange parameters include net photosynthesis rate, stomata conductance, intercellular CO_2, transpiration rate, and leaves vapour pressure deficit, survival rate, plant height and diameter, plant biomass, and also from the leachate chemical analysis.

1.2 Problems statements and Justification

The uses of sewage sludge as commercial fertilizer in agricultural soils nowadays may resulting the contaminants of groundwater by heavy metals. Rosenani et. al (2004) reported that sewage sludge in Malaysia contains heavy metals such as Cu, Pb, and Zn. Wong et. al (2001) also stated that sewage sludge is a major source of heavy metals containing Zn, Pb, and Cu. Theseheavy metals willflow through the porous medium into groundwater and may cause health problems to all living things.Since a priority agenda in many world forums nowadays focus on phytoremediation currently is increasing, a potential plant must be used to remove the contaminant sites. Numerous studies indicated that many plant species have been tested because of their ability to accumulate toxic elements (Tlustos et al., 2006), but little research has been directed towards determining the physiological performance and growth responses of *M. cajuputi* by a simple lysimeter method.

In the other hand, heavy metal contaminants in the solid state likesewage sludge as treatment in an experiment, may not shown clearly how the specific heavy metal, zinc (Zn) and copper (Cu)will be absorbed by plant instead of using liquid state of these heavy metal directly as treatment. Plant will directly pump the selected heavy metals that have been dissolved in water at certain level concentration. Besides,Hilber in 2007 (as cited in Basta et al., 2005) stated that zinc (Zn) and copper (Cu) will bound to the organic matter and to Aluminium (Al) and Iron (Fe) oxides in sewage sludge. Apart of that, different heavy metal concentrations may cause different growth responses in *M. cajuputi*. Even though, both of these selected heavy metals, Zn and Cu have essential values for plant growth and physiological performances but the sufficiently high concentration of these heavy metals can become toxic and give negative feedback to all living things. In addition, previous study had proved that copper and zinc will become toxic when they exceed a maximum soil concentration 125 mg/kg and 400 mg/kg respectively (Rossi et al., 2004).Madyar (2008) stated that Zn and Cu are considered dangerous for organisms at concentrations of 5 and 1 mg/L, respectively.

The lysimeter study is not something new for the whole world but in our country, it is less practically done. Since this lysimeter study gained more and more importance nowadays, we have to expose this new technology method to our society. This is one of friendly and cheapest way to study the increasing pollution and contamination of groundwater problems in our country recently. Based on Lazarovith (2006), it is also an important tool in soil-plant-atmosphere research nowadays since it can directly measure the actual amount of evapotranspiration (ET) rate and facilitate water, fertilizer, and solute balance studies.

1.3 Objectives

1) Todetermine thephysiological and growth performances of *Melaleuca cajuputi*as phytoremediator of heavy metal elements of Copper and Zinc.

2) To investigate the best concentration level of both Copper and Zinc for plant physiological attributes and tolerance that planted in a lysimeter model.

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