ACCUMULATION OF ZINC BY *Alternanthera sessilis*

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Science

Department of Bioscience
Faculty of Science
Universiti Teknologi Malaysia

APRIL 2020

DEDICATION

Specially dedicated to my beloved parents,
My supervisor Dr.Zaidah Rahmat and co-supervisor Dr. Fazilah Abd Manan,
All my family members, uncles and aunties and all my precious friends,
For their patience in my effort as a student,
For whom I am,
And the achievement that I have made.

ACKNOWLEDGEMENT

First and foremost, all thanks to Lord Muruga for giving me courage, guidance, power and wisdom to successfully complete this project in Universiti Teknologi Malaysia. I would like to express my sincere gratitude and deepest appreciation to my Final Year Project's supervisor, Dr. Zaidah Rahmat and co-supervisor, Dr.Fazilah Abd Manan for their valuable advices, consistent guidance and encouragement leading to the completion of this study.

Special thanks to my seniors, juniors and lab assistant from Wastewater Research Laboratory for assisting my experiment preparations. Not forgetting my friends from Department of Biosciences (Pavitra Nandagopal, Siti Mariam Binti Mohamad, Song Yu Qing and Jonathan Goh Jia En) who gave me shoulder and support throughout the research journey. I also want to acknowledge my greatest appreciation especially to my lovely parents and uncles for their support, understanding and encouragement during my research all long the journey.

Last but not least, I wish to acknowledge the support from Universiti Teknologi Malaysia for National Postgraduate Fund scholarship for every semester.

ABSTRACT

Zinc is an essential element that contribute to plant growth and developmental processes and induces tolerance mechanism to adapt the external environment. However, excess amount of zinc in soil become pollutant to the environment. In Malaysia, especially in Johor, zinc accumulation is one of the major problems as soil contaminant cause long term effect to the environment. Fortunately, there are plants that could tolerate and accumulate high amount of zinc from soil known as zinc hyperaccumulators. *Alternanthera sessilis* is known as a hyperaccumulator for chromium and potential hyperaccumulator for zinc. This study was carried out to determine the ability of *A. sessilis* in up taking different zinc concentrations (0 ppm, 50 ppm, 100 ppm, 200 ppm and 300 ppm) and the compartmentalization of zinc in different parts of the plant. Overall growth of *A. sessilis* at different zinc concentrations were observed and analysed by statistical analysis to determine morphological changes in plant growth. Plant samples were extracted by acid digestion method and analysed by inductively coupled plasma-optical emission spectroscopy (ICP-OES) analysis to determine zinc concentrations in each part of the plant sample. The result of this study indicates that *A. sessilis* morphological growth and development was established as common growth phases from vegetative stage to branching stage with an estimation of two months and 15 days is suitable transfer to zinc treated soil. *A. sessilis* shows healthier growth in terms of new leaves, branches, height of the plant and roots at all zinc concentrations tested with no significant difference among treatments except for root length. The roots of *A. sessilis* at 0 ppm treatment showed significantly higher zinc accumulation (1.43 ± 0.01 ppm) and significantly lower zinc accumulation in stems of 0 ppm treatment (0.20 ± 0.00 ppm). The higher total zinc uptake by the *A. sessilis* at 0 ppm (control) treatment (2.04 ± 0.03 ppm) and the lower observed at 300 ppm treatment (1.26 ± 0.00 ppm). The total zinc accumulation in *A. sessilis* from various concentrations of zinc treatment were in the order of 0 ppm < 50 ppm < 100 ppm and 200 ppm < 300 ppm zinc concentrations. *A. sessilis* has the potential to hyperaccumulate zinc which may help to reduce zinc in the soil and clean up zinc in polluted environment.

ABSTRAK

Zink adalah elemen yang penting kepada tumbuh-tumbuhan untuk proses pertumbuhan dan tumbesaran dan mendorong mekanisma toleransi untuk menyesuaikan diri dengan persekitaran luaran. Walau bagaimanapun, jumlah zink yang berlebihan dalam tanah menjadi pencemar kepada alam sekitar. Di Malaysia, terutamanya Johor, pengumpulan zink berlebihan adalah salah satu masalah utama sebagai bahan pencemar tanah yang menyebabkan kesan jangka panjang kepada alam sekitar. Mujurlah, terdapat tumbuh-tumbuhan yang mempunyai toleran dan mengumpul sejumlah zink yang banyak daripada tanah dikenali sebagai *zinc hyperaccumulator*. *Alternanthera sessilis* dikenali sebagai *hyperaccumulator* untuk kromium dan berpotensi sebagai *hyperaccumulator* untuk zink. Kajian ini dijalankan untuk menentukan keupayaan *A. sessilis* tumbuh pada kepekatan zink yang berbeza (0 ppm, 50 ppm, 100 ppm, 200 ppm dan 300 ppm) dan pengumpulan zink dalam bahagian pokok yang berbeza. Perubahan morfologi *A. sessilis* yang dirawat pada kepekatan zink yang berbeza telah diperhatikan dan ditentukan oleh analisa statistik. Sampel pokok telah diekstrak melalui pencernaan asid dan dianalisis dengan analisis inductively coupled plasma-optical emission spectroscopy (ICP-OES) untuk menentukan jumlah zink di dalam setiap bahagian pokok tersebut. Hasil kajian ini menunjukkan, bahawa pertumbuhan morfologi and tumbesaran *A. sessilis* sebagai pertumbuhan biasa dari peringkat vegetatif ke peringkat cabang yang mengambil masa anggaran selama dua bulan dan 15 hari dan sesuai sebelum pokok ini dipindah ke tanah yang dirawat dengan zink. Pertumbuhan *A. sessilis* lebih sihat dari segi pengeluaran daun baru, cabang, ketinggian pokok dan akar di dalam semua kepekatan zink dalam tanah yang diuji dan tidak menunjukkan perbezaan yang signifikan kecuali panjang akar. Akar *A. sessilis* pada 0 ppm (kawalan) menunjukkan pengumpulan zink dengan signifikan tinggi (1.43 ± 0.01 ppm) dan pengumpulan zink dengan signifikan rendah di batang 0 ppm (0.20 ± 0.00 ppm). Secara keseluruhan, pengumpulan zink oleh *A. sessilis,* tertinggi diperhatikan pada 0 ppm sebanyak 2.04 ppm dan pengumpulan zink terendah diperhatikan pada 300 ppm sebanyak 1.26 ppm. Jumlah pengumpulan zink oleh *A. sessilis* pada kepekatan zink yang berbeza dalam tanah disusunkan dalam urutan 0 ppm < 50 ppm < 100 ppm and 200 ppm < 300 ppm. *A. sessilis* mempunyai potensi untuk mengumpul zink yang banyak dan ia membantu mengurangkan zink dalam tanah dan membersihkan zink daripada persekitaran yang tercemar.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CDF	-	Cation diffusion facilitator
CPD	-	Critical point dryer
CSQG	-	Canadian soil quality guidelines
EDXRF	-	Energy dispersive x-ray flouresence
FRIM	-	Forest research institute Malaysia
FS	-	Faculty science
HDL	-	High density lipoprotein
HT-29	-	Human colorectal adenocarcinoma cell line
ICP-OES	-	Inductively coupled plasma-optical emission spectroscopy
MCF-7	-	Michigan Cancer Foundation-7
NPK	-	Nitrogen, phosphorous and potassium
RDA		Recommended daily allowance
SBID		Plant sample code
SEM		Scanning electron microscopy
UIRL		University internal research laboratory
UTM		University technology Malaysia
ZIP		ZRT-IRT like proteins

LIST OF SYMBOLS

G	-	Gram
mg / day	-	Milligram per day
mg / kg	-	Milligram per kilogram
mg / L	-	Milligram per litre
µg / g	-	Microgram per gram
mL	-	Millilitre
Ppm	-	Part per million
Mm	-	Millimetre
Cm	-	Centimetre
°C	-	Degree Celsius
v / v	-	Volume per volume
%	-	Percentage
pH	-	Potential of hydrogen
p	-	Probability
>	-	Greater than
<	-	Less than
±	-	Plus minus
$Kr_2Cr_2O_7$	-	Potassium dichromate
$ZnSO_4.7H_2O$	-	Zinc heptahydrate
ATPase	-	Adenosine triphosphatase
K	-	Potassium
Ca	-	Calcium
Fe	-	Iron
Cu	-	Copper
N	-	Nitrogen
P	-	Phosphorous
Na^+	-	Sodium ion
K^+	-	Potassium ion
Zn^{2+}	-	Zinc ion

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Zinc is an essential heavy metal for plants including *Alternanthera sessilis*. It acts as micronutrient for plants growth and developmental processes. It is necessary for plants to regulate tolerance mechanism via gene expression against environmental changes (Sidhu, 2016). Zinc has greater mobility in plants that occurs rapidly as Zn^{2+} ions or by forming complex with organic ligands through xylem and translocate to upper parts of plants while the distribution is depends on the zinc concentration in plants (Sidhu, 2016). There are two types of main zinc transporters available in plants such as ZIP (ZRT-IRT like proteins) transporter family, CDF (Cation Diffusion Facilitator) family that transports zinc from environment to the plants via metal efflux (Sidhu, 2016). All over the world more than 400 plant species are categorised as zinc accumulator and among this, 16 plants are known as zinc hyperaccumulating plants including *A.sessilis* (Sidhu, 2016).

Alternanthera sessilis is a globally distributed invasive type of weedy plant and it is no longer considered as native plant because of increasing population trend (CABI, 2018) (www.cabi.org/ISC). The habitat of *A. sessilis* are terrestrial and freshwater system, where it can adapt and grow well in different environmental conditions. This plant is commonly found in gardens, roadsides, pathways, wasteland, irrigation canals, wetlands, swamps, ditches, dykes, and fallow ground (Lansdown and Beentje, 2017). In Malaysia, *A. sessilis* is known by many names such as akar rumput, bayam pasir, bayam tanah, carpet weed keremak, kelama hijau, kerak-kerak paya, keremak and kerumak bukit paya (CABI, 2018) (www.cabi.org/ISC). *A. sessilis* is able to reproduce asexually by either seeds or vegetatively by stems. The seeds of *A. sessilis* is dispersed by wind and water while the stem able to produce new shoots once it was established.

The stages of growth of this plant starts from flowering, fruiting, post-harvest to seedling stage (CABI, 2018) (www.cabi.org/ISC). Although it is a small weedy plant, it able to grow very fast hence enabling translocation from one place to another faster while utilizing larger land area (Mazumdar and Das, 2015).

A. sessilis grows well on wetlands and it able to tolerate extreme condition and acts as an accumulator that has been proven by some studies. According to Subhalakshmi *et al.*, (2013), *A. sessilis* is a claimed hyperaccumulator for chromium and is able to trap more heavy metal above the threshold level (500 to 3400 μg / kg or ppm) (Kabata-pendias, 2000). Mazumdar and Das (2015) reported that *A. sessilis* has the potential to work as a hyperaccumulator since it fulfils all the basic criteria as wetland plant species and additionally have the high translocation factor that enables it to trap high amount of heavy metals including zinc from roots through shoots and leaves. In future, these phytoremediation process by *A. sessilis* may aid phytomining for recovery of heavy metals from polluted area.

Zinc is an essential micronutrient but excess amount of zinc in soil cause harm to the environment. Accumulation of zinc in soil become threats for farmlands (Alloway, 2008). It affects plant growth on the zinc rich soils and only highly zinc tolerance plants are able to survive under this condition and these kind of plants are known as hyperaccumulators (Alloway, 2008; Baran *et al.,* 2018). Furthermore, zinc rich farmlands also affects dairy animals such as cattles, goats which ate grasses and some other plants as their main food source from this area. The zinc rich plant food source causes health damage to the animals due to it enters the food chain (Alloway, 2008; Jiang *et al.,* 2011). Apart from animals and plants, microbes also affected by zinc rich soils as the microbial activities interrupted such as slow down or of organic matter breakdown due to underground environmental changes (Alloway, 2008). The accumulation of zinc also affects earthworms and other insects lives in soil (Alloway, 2008; Pochron *et al.,* 2017). Hence, the excess amount of water soluble zinc in soil may contaminate groundwater system by leaching of the soil (Zheng *et al.,* 2012) and possibly to mixed with waterways and cause harm to the coastal marine system (Inui and Katsumi, 2019).

1.2 Problem Statement

Zinc accumulation is one of the major problem in Malaysia affecting water and soil that cause harm to the environment (Baharom and Ishak, 2015; Ong *et al.*, 2016; Ismail *et al.*, 2017). In Johor specifically at Kampung Pasir Puteh, due to industrialization and urbanization, domestic wastes such as organic wastes rich in zinc causes zinc accumulation on sediments and soil (Suhaimi-Othman *et al.*, 2012). Zinc accumulation in soil cause long term effect and become a threat to the environment and human health when it enters into the food chain (Suhaimi-Othman *et al.*, 2012; Maadin *et al.*, 2016). Phytoremediation is an alternative method to remediate or reduce zinc concentration in soil due to the plant's ability to trap heavy metals. According to a previous study, *A. sessilis* is a potential hyperaccumulator for plumbum, zinc, ferrous and magnesium (Mazmudar and Das, 2015). But until now, only limited number of research or study had been done on analyzing the ability of *A. sessilis* in up-taking zinc (Mazmudar and Das, 2015; Suthari *et al.*, 2017). If *A. sessilis* is able to accumulate zinc in soil, it would help in agricultural applications as the plant grows faster than food crops. This would reduce zinc absorption in food crop and could be safer for human consumption.

1.3 Research Objectives

The objectives of the research are:

(a) To determine the morphological growth and development stages of *A. sessilis*.

(b) To determine accumulation of zinc in different part of the plant (leaf, stem, root).

1.4 Significance of Study

A. sessilis is an invasive plant suggested for phytoremediation activity as it is able to grow fast with high tolerance to a variety of environment and able to accumulate high amount of heavy metals including zinc (Mazumdar and Das, 2015; CABI, 2018)(www.cabi.org/ISC). This plant has high translocation factor that enhance the ability to transfer heavy metal especially zinc from one plant part to another efficiently. *A. sessilis* has a tappy root system that may become another reason for increasing zinc accumulation into plant (Mazumdar and Das, 2015). Hence, phytoremediation by *A. sessilis* may be suitable to extract zinc faster and reduce zinc accumulation in the soil to clean up zinc in polluted environment.

1.5 Scope of Study

The focus of this research started with observation of *A. sessilis* growth at different growth stages followed by identifying the suitable stage for induction of different concentration of zinc in the soil. The plant was grown for 21 days after the soil being induced with zinc. Then, morphological characteristic of *A. sessilis* was studied. Zinc accumulation in different parts of *A. sessilis* such as leaves, stem and roots were determined to know maximum zinc uptake by using inductively coupled plasma-optical emission spectroscopy (ICP-OES) analysis via acid digestion.

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