EFFECT OF ZINC AND SILVER ON THE GROWTH OF *Pleurotus ostreatus*

FATIMA MOHAMED YOUSIF

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
BACHELOR OF BIOTECHNOLOGY (HONOURS)

FACULTY OF HEALTH AND LIFE SCIENCES
INTI INTERNATIONAL UNIVERSITY
PUTRA NILAI MALAYSIA

January 2018

NON-PLAGIARISM DECLARATION

By this letter I declare that I have written this dissertation completely by myself, and that I have used no other sources or resources than the ones mentioned.

I have indicated all quotes and citations that were literally taken from publications, or that were in close accordance with the meaning of those publications, as such. All sources and other resources used are stated in the references.

Moreover I have not handed in a dissertation similar in contents elsewhere.

In case of proof that the dissertation has not been constructed in accordance with this declaration, the Faculty of Health and Life Sciences has the right to consider the dissertation as a deliberate act that has been aimed at making correct judgment of the candidate's expertise, insights and skills impossible.

I acknowledge that the assessor of this item may, for the purpose of assessing this item,

- reproduce this assessment item and provide a copy to another member of the University; and/or,
- communicate a copy of this assessment item to a plagiarism checking service (which may then retain a copy of the assessment item on its database for the purpose of future plagiarism checking).

In case of plagiarism the examiner has the right to fail me and take action as prescribed by the rules regarding Academic Misconduct practiced by INTI International University.

FATIMA MOHAMED YOUSIF	
Name	Signature
I15007606	20th April 2018
I.D .Number	Date

DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged, and completed under the supervision of Dr. Thong Weng Hing

Fatima Mohamed Yousif	*Dr. Thong Weng Hing*
I.D. NUMBER: I15007606	(SUPERVISOR)
DATE: 20th April 2018	

ACKNOWLEDGEMENT

First of all, I would like to pay my praises and humblest thanks to God, the most merciful and beneficiate of all, who bestowed me with the ability and health to complete this project. Secondly, for the most important man in my life; my father, who supports, motivates and believes on me always. Deep thanks to my beloved mother and all my family members for the unlimited love and encouragement that helped me through this tough journey. Their prayers for my success have always been the pillar of my strength in life.

Next, I would appreciate and like to express my gratitude to my supervisor, Dr. Thong Weng Hing, for the guidance and advises throughout this journey to complete my project. And to my mentor, Dr. Wong Kok Kee, for his valuable and stimulating suggestions and encouragement which helped me in all the times.

Also, big thanks to INTI International University for giving me the opportunity to conduct this project as is gave me an astronomic knowledge. I consider myself as a very lucky individual as I was provided with an opportunity to be part of it. I am also so grateful for having a chance to meet so many wonderful people and professionals who led me through my Bachelors period.

ABSTRACT

Pollution is the addition of something that does not naturally exist to the ecosystem. Heavy metals pollution has become a global concern as it imposes a lot of negative impacts on living organisms, human, animals and plants. Mycoremediation is the process of using fungi in order to remove contamination from the environment. The objective of this study was to determine the effect of zinc (Zn) and silver (Ag) on the growth of *Pleurotus ostreatus*. In this study, the mycelia of *P. ostreatus* was cultured on PDA medium containing different concentrations of Zn or Ag ranging between 1 mg/L to 200 mg/L. The optimum growth obtained by *P. ostreatus* in Zn plates was 4.2 cm at 5 mg/L and for Ag was 4.2 cm at 1 mg/L and 5 mg/L. It was noticed that Ag exerted higher toxicity towards *P. ostreatus* compared to Zn as the growth for Ag plates was slower than Zn plates. *P. ostreatus* was able to serve as a potential model for mycoremediation as *P. ostreatus* had the ability to grow and tolerate high concentrations of heavy metals namely Zn and Ag.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

°C	Degree Celsius: Unit of temperature
P. ostreatus	*Pleurotus ostreatus*
ANOVA	Analysis of Variance
Zn	Zinc
Ag	Silver
mL	Milliliter: Unit of volume
mg	Miligram: Unit of concentration
mgL^{-1}	Miligram per Liter: Unit of concentration
%	Percentage
PDA	Potato Dextrose Agar
d	Days
Min	Minutes
g	gram
PAHs	Polycyclic aromatic hydrocarbons
ANOVA	Analysis of Variance
dH_2O	Deionised water
cm	Centimetre: Unit of length

CHAPTER 1

INTRODUCTION

Pollution is the introduction of contaminant to the natural environment or the addition of something that does not naturally exist to the ecosystem (Baig, 2012). These foreign materials that has been introduced into the environment are known as pollutant (Baig, 2012). Pollution can occur by natural catastrophe but most of the times is caused by human actions (Commoner et al., 2010). Small actions of ours such as smoking, the uses of pesticides or insecticides, washing clothes near to the lake by some people in slum areas and the gases produced from air conditioners and refrigerators such as hydrofluorocarbons can cause contamination to our environment (Commoner et al., 2010). Pollution has become a global concern as it impose a lot of impacts on living organisms, human, animals and plants. The adverse effect of pollution on human can lead to death because it might affect the lungs functions, and cause cancer due to the presence of carcinogenic contents (Singh et al., 2011). Besides, water pollution can cause damage to the liver and kidney. Water pollution will lead to suppresses tadpole and frog biodiversity (Singh et al., 2011). Moreover, industrial pollution can negatively affect the growth of plants and aquatic organisms due to the formation of acid rain in the air (Singh et al., 2011).

Heavy metals are metallic chemical elements that has a relatively high density. They are naturally occurring and have high atomic weight five times greater than that of water (Anahid et al., 2011). Living organisms need varying amount of heavy metals such as zinc (Zn), cobalt (Co), manganese (Mn), iron (Fe), copper (Cu) and molybdenum (Mo) as they help in the process of metabolism of living organisms (Anahid et al., 2011). Heavy metals enter our bodies via water, food or air. However, heavy metals turn to be toxic and detrimental to living organisms at high concentration (Anahid et al., 2011). On the other hand, metals such as plutonium (Pu), lead (Pb) and mercury (Hg) are toxic metals which have no benefits for organisms and their accumulation over time can lead to serious illness (Anahid et al., 2011).

Accumulation of heavy metals in plants can lead to cellular damage and disturbance of cellular ionic homeostasis (Famalicao, 2010). However, plants have developed detoxification mechanisms based on subcellular and chelation compartmentalization to reduce the effects of heavy metals (Famalicao, 2010). Heavy metals could be adversely toxic

and limit the growth of fungi (Famalicao, 2010). The increases of industrial activity caused the increased of demand for heavy metals such as Zn, Hg, Mn, Pb and Cu which in turn lead to the increase of global quantity of heavy metals contaminated wastewater (Famalicao, 2010).

Few technologies have been used to remove metals in low concentration from waste water (Rhodes, 2014). Processes like ion exchange and precipitation are effective but expensive. Scientist focused on the study of bioaccumulation and biosorption in fungi for metal removal as it is inexpensive and sufficient system (Rhodes, 2014). Mycoremediation is the process of using fungi in order to remove contamination from the environment by releasing of extra-cellular lignin-modifying enzymes, with a low substrate-specificity, as they can act upon different molecules that are commonly similar to lignin (Rhodes, 2014). One of the major advantages of mycoremediation is low cost as it uses fungi. *Pleurotus ostreatus* known to be a successful model in cleaning up pollutant in soil (Sykes, 2002). *P. ostreatus* is able to degrade polycyclic aromatic hydrocarbons (PAHs) by up to 97% and it has been shown that fungi can degrade complex PAHs with up to 6 benzene rings (Cerniglia, 1997). *P. ostreatus* is a common oyster mushroom that spread throughout the world. It grow faster and have many varieties of oysters. It grows on a wide range substrates such as corn, coffee and rice. Scientists prefer to work with *P. ostreatus* as it is easy to be cultured in the laboratories (Tisdale et al., 2005).

There are a lot of experiment have been done on the effect of heavy metals such as Mn, Pb, Hg, Fe and Cl on the growth of *P. ostreatus*. To date, there is no study done on the effect of Zn and Ag on the growth of *P. ostreatus*. Therefore, the aim of this study is to determine the effect of Zn and Ag on the growth of *P. ostreatus*.

CHAPTER 2

LITERATURE REVIEW

2.1 ENVIRONMENTAL POLLUTION

Pollution is the addition of any material liquid, gas or solid or any energy form sound, heat or radioactivity to the environment in faster rate than the normal (Baig, 2012). Pollution has been classified into three major groups, water pollution, land pollution and air pollution. While modern society is worried about special types of pollution such as light pollutant, noise pollutant and plastic pollutant (Baig, 2012).

The pollutants in living organisms can cause mild to serious diseases such as cancer (Sim, 2011). Air pollution can reduce lung function in human and increases the chances of getting asthma attacks (Sim, 2011). Water pollution can cause typhoid, hookworm and amoebiasis. In addition, soil pollution can cause cancers such as leukemia (Sim, 2011).

2.1.1 HEAVY METALS POLLUTION

Heavy metals are naturally occurring metallic chemical elements that relatively have high density and high atomic weight five times greater than that of water (Anahid et al., 2011). Heavy metals are usually toxic at low concentration and it has been recorded that a little amount of heavy metals can become toxin and cause side effects to living organisms (Martin, 1993). There are few factors that the toxicity of metal depends on including route of dosage exposure, age, gender, chemical species and genetics (Barakat, 2011). Researchers have found that water polluted by heavy metals can cause cancer, hormonal problems, DNA, kidney and liver damages (Singh et al., 2011).

Metals like zinc (Zn), lead (Pb) and copper (Cu) are the causes of environmental pollution from sources such as industrial effluents and leaded petrol (Anahid et al., 2011). Heavy metals are dangerous as they tend to be bioaccumulated organism's body over time (Mathew & Krishnamurthy, 2015).

Even though heavy metals can be dangerous to living organisms, but at the same time they have some positive impacts (Martin, 1993). All living organisms need different amount of heavy metals such as Fe, Cu, Zn and Mn as it needed by living organisms for metabolism

processes (Martin, 1993). As an example, Cu at lower concentration performs as co-factors for different enzymes of redox cycling (Draszawaka, 2014).

2.2 ZINC (Zn)

Zinc is a metal that belongs to group 12 of the periodic table. Zn is an essential trace element which impose biological importance for animals and plants (Draszawaka, 2014). Zn helps to stimulate the activity of more than 100 different enzymes in human body, regulate immune function, treating for diarrhoea and common cold and plays an important role in wound healing (Mielcaez et al., 2017). Zn can be naturally found in different types of food as beans, animal meats, seafood and whole grain cereals or can be taken as a dietary supplement (Plum et al., 2010). However, 2-12 mg (depending on the age) of zinc is needed to make a person sensitive to illness and disease (Draszawaka, 2014).

Zn is one of the important micronutrients needed by plants in small amount (Draszawaka, 2014). It helps in the process of internodes elongation and growth hormone production. Zn deficiency can result in reducing crops quality and yield by over 20% (Zhao et al., 2012). Symptoms of Zn deficiency in plants are brown spots on upper part of leave, interveinal chlorosis and leaves may be totally distorted (Tsonev & Lidon, 2012). Zn deficiency can be caused by low level of Zn in soil, high soil pH and low soil temperature (Tsonev & Lidon, 2012).

The negative impact of Zn on fungal communities are concerned. It was reported that 57 mg Zn in 1g of soil is the mean value of Zn in the natural environment (Babich & Stotzky, 1978). Above this concentration indicate toxicity and can have serious effects on soil organisms such as oyster mushroom (Babich & Stotzky, 1978).

2.3 SILVER (Ag)

Silver is a naturally occurring element that belongs to group 11 of the periodic table. Pure silver has the maximum thermal and electrical conductivity of all metals (Durham, 2013). Metallic silver is used for splints, surgical prosthesis and coinage and silver salt is used in treating epilepsy, mental illness and infection disease such as gonorrhoea (Durham, 2013).

Unlike Zn, Ag have no biological role and it is harmful to living organisms at very low concentrations (Gruyer et al., 2013). High doses of Ag to human might lead to breathing

problems, liver damage, skin damage and kidney damage. Scientists have found that silver nanoparticles have a significant impact on the growth of plants and fungi (Kim et al., 2012).

2.4 BIOREMEDIATION

The word remediate refers to solve a problem, and bio-remediate means the uses of biological organisms in order to solve environmental problems such as contaminated groundwater or soil. In non-polluted environment, protists, bacteria, fungi and microorganisms are regularly breaking down the organic matter (Markykensa, 2011).

Bioremediation can be classified into two groups, *in situ,* which means the process involving treatment of contaminated material at the site whereas *ex situ*, is the elimination of contaminated matter to be handled away from the site (Hussain, 2016). Scientists have focused on the study of bioremediation as it require low cost due to the uses of microorganisms, fungi and plants, and less equipments are required compare to other technologies. Besides, bioremediation does not use any chemicals to cleanup pollutants thus, it does not introduce more chemicals to our environment (Karigar & Rao, 2011).

2.4.1 MYCOREMEDIATION

Mycoremediation is a type of bioremediation, it uses fungi to cleanup pollutants from the environment (usually soil). The term mycoremediation was formulated by Paul Stamets to indicate the uses of fungal mycelia in bioremediation (Rhodes, 2014). The advantages of mycoremediation are safe, natural, low cost, reusable and fast (Rhodes, 2014).

Fungi have an important decomposition in ecosystem (Sykes, 2002). They have the ability to breakdown complex molecules and long chain toxin to simpler less toxic chemicals (Kulshereshtha et al., 2014). Moreover, fungi can extract heavy metals by transporting them into their fruit bodies. The mycelium excretes extracellular acids and enzymes that breakdown cellulose and lignin, the two important building blocks of plants fibre (Kulshereshtha et al., 2014).

2.5 *Pleurotus ostreatus*

P. ostreatus is a common oyster mushroom that spread throughout the world. It grows fast and has many varieties. It is considered as one of the most easily cultivated and fast growing fungi in the world. *P. ostreatus* is white-rot fungus that spread widely in America, Asia, Europe, Australia and Northern Africa. *P. ostreatus* have large fruit bodies (up to 20 cm),