EFFECT OF CADMIUM AND COPPER ON THE GROWTH OF *Pleurotus ostreatus*

TAN SHU RONG

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

2017

NON-PLAGIARISM DECLARATION

By this letter I declare that I have written this thesis 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.

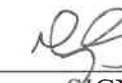
Moreover, I have not handed in a thesis similar in contents elsewhere.

In case of proof that the thesis has not been constructed in accordance with this declaration, the Faculty of Health and Life Sciences has the right to consider the research thesis 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.

Tan Shu Rong	
NAME	SIGNATURE
I14004744	20th November 2017
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

Tan Shu Rong	*Dr. Thong Weng Hing*
I.D. NUMBER: I14004744	(SUPERVISOR)
DATE: 20th November 2017	
	(CO-SUPERVIOSR if any)

ACKNOWLEDGEMENT

First and foremost, I would like to thank God for his never-ending grace, mercy, and provision during what ended up being one of the toughest times of my life for completing this final project. During the 10 weeks of laboratory work, I faced much problems in my research but thanks to my classmates which they always gave support and assist to me. Throughout this journey, they always cherishing and comforting me. They are my most value person which give me encourage and motivation for completing this project.

Next, I would like to take this opportunity to make sincerely thanks to my supervisor which is Dr. Thong Weng Hing who gives me valuable guidance and expertise from the beginning till the end to complete my final project. I also want to thank Dr. Ong Ghim Hock for teaching me doing ANOVA analysis as well other lecturer for their guidance and support throughout my work. Then, I like to express my sincere acknowledgement to INTI University, Nilai campus and laboratory staff as well which allow me to proceed my final project successfully by providing the proper laboratory, materials and advanced equipment. Not forgetting my senior, Wee Ing Kian who provide me countless help and motivation during this project.

Last but not least, I would like to thank my family for being my pillar of strength providing motivational, emotional and financial in order for me to complete this project.

ABSTRACT

Recently, heavy metals pollution has become a global issue. Many studies showed that heavy metal at high concentrations could result in toxicity in living organisms. Heavy metals such as cadmium (Cd), arsenic (As), mercury (Hg) and lead (Pb) cause toxicity even at low concentration. Mycoremediation could be the solution for heavy metal contamination in the environment. *Pleurotus ostreatus* is a good candidate to remove heavy metal because it has high viability, high tolerance towards temperature and high adsorption towards heavy metal. The objective of this study was to determine the effect of cadmium (Cd) and copper (Cu) towards the growth of *P. ostreatus*. In this experiment, the spawn of *P. ostreatus* was cultured on the centre of the potato dextrose agar (PDA) medium and PDA media supplemented with nine different concentrations of Cd or Cu (1, 5, 10, 20, 50, 100, 150, 200, 400 mg/L). In this study, the optimum growth of *P. ostreatus* in PDA media containing Cd was achieved at 1 mg/L Cd whereas the lowest growth was produced at 400 mg/L with the mean of radius as 4.38 cm and 0.88 cm, respectively. On the other hand, PDA medium supplemented with 5 mg/L Cu produced the optimum mycelial growth while 400 mg/L Cu showed the lowest mean of mycelial radius. It was noticed that Cd exerted higher toxicity at elevated concentrations towards *P. ostreatus* than Cu. *Pleurotus ostreatus* is tolerant to certain level of heavy metals and could serve as an agent of mycoremediation.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Cd	Cadmium
Cu	Copper
P. ostreatus	*Pleurotus ostreatus*
°C	Degree Celsius: Unit of Temperature
As	Arsenic
Be	Beryllium
Pb	Lead
Hg	Mercury
Fe	Iron
Zn	Zinc
Ni	Nickel
%	Percentage
pH	power of hydrogen: Measure of concentration of hydrogen ion
PDA	Potato Dextrose Agar
g	gram: Unit of concentration
mL	Mililiter: Unit of Volume
kPa	Kilopascal: Unit of pressure
Min	Minute
$mgmL^{-1}$	Miligram per Mililiter: Unit of concentration
mgL^{-1}	Miligram per Liter: Unit of concentration
mg	Miligram: Unit of concentration
ANOVA	Analysis of Variance
$Cd(NO_3)_2$	Cadmium nitrate

$CuSO_4$	Copper(II) sulfate
dH_2O	Deionised water
cm	Centimetre: Unit of length
µg/g	Microgram per Gram: Unit of mass
A. niger	*Aspergillus niger*
LSD	Least Significant Difference

CHAPTER 1

INTRODUCTION

Pollution has become a global issue nowadays. What is pollution? The term of pollution can be defined as undesirable changing of the characteristics of air, water and soil in their physical and chemical properties which can create harmful effect to the environment and create health hazard which may affect the health of the living organisms on the earth (Pollution, 2004). According to Coker, (1990), pollution can be classified into different types such as air pollution, water pollution, land pollution as well as noise pollution. The most common pollutants which resulted in pollution are pesticides, heavy metals and hydrocarbons (Slattery, 2012).

Heavy metal pollution is a type of pollution caused by heavy metals. Heavy metal can be defined as a specific metallic element which has very high density and they are usually toxic or poisonous agents even at very low concentration (Duruibe, Ogwuegbu, and Egwurugwu, 2007). There are many types of heavy metals, such as Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb) and Zinc (Zn) which all of these are major concern of the environment pollution research (Heavy metal pollution, 2011). Heavy metals are able to bring many problems to the organisms as it is toxic and can affect the physiological functions, mortality, growth rates and reproduction of living organisms. Besides, heavy metals can be accumulated in the food source and cause bio-magnification throughout the food chain (Afshan, *et al.* 2014).

Cadmium is widely used in coating, batteries, plating, alloys as well as in many other industries. Cd can be hazardous to both environment and human (Sharma & Rawal, 2016). Cadmium adversely affects our health even at very low concentration causing renal tubular dysfunction, formation of kidney stone, disturbance in calcium metabolism and maybe cancer. In the worst case, death would be resulted (World Health Organization, 2010). Cadmium can be found in water, atmosphere and even in food. Cadmium is naturally found in the ocean water, earth's crust and volcano whereas mining, combustion of fossil fuels, waste incineration, smelting of zinc-bearing ores and release from municipal landfills are the anthropogenic source of Cd (Cadmium and cadmium compounds in plastics, 2012).

Copper (Cu) another heavy metal that is highly concerned besides Cd. Copper is widely used in many different types of products especially electrical products due to their higher rate of electrical and thermal conductivity (Copper Development Association, 1998). Major sources of Cu pollution are mining operations, corrosion of copper pipes used in home plumbing, sewage treatment plant sludges and burning of coal in power plants. Copper is toxic to the aquatic organisms, especially the fish as they are very highly sensitive towards the copper. In Tsolum River on Vancouver Island, the copper contamination is very serious due to the copper mining until the whole population of salmon was almost wipe out (Copper fact sheet, 2002). In addition, Cu can bring harmful effect towards human health too. Copper would result in temporary gastrointestinal distress together with the symptoms such as vomiting, nausea and abdominal pain in short term exposure while causing damage towards liver and kidney in long term exposure. One good example of long-term effects is Wilson's disease which is an inherited genetic disorder resulted by accumulation of Cu in liver. Besides, Cu is carcinogenic to human too, several studies have shown that worker that exposed to Cu has higher chance to get cancer (Copper: health information summary, 2013).

Bioremediation has been applied to reduce heavy metal pollution. One of the technique is bioaccumulation. Bioaccumulation is a process of accumulating pollutants in the body of a living organism, thus, reducing the pollutants in the environment (Tsekova, Kaimaktchiev, and Tzekova, 1998). Fungi is commonly used in the process of bioaccumulation of heavy metal as they have very high tolerance in heavy metal in addition to their greater biomass, aggressive growth, high production and extensive hyphae to reach out into soil (Iram, *et al.*, 2009). *Pleurotus ostreatus* also known as oyster mushroom could be used as an agent of bioaccumulation to remediate pollutants such as Cd and Cu (Das, 2005). *Pleurotus ostreatus* are edible fungi which commonly found in south east Asia, India, Europe and Africa. It grows widely in the tropical and subtropical rainforest. These mushrooms can be used industrially for mycoremediation purposes. Hence, its cultivation can play an important role in managing organic wastes whose disposal has become a problem (Oyetayo & Ariyo, 2013). Cadmium able to accumulate in the subcellular cytosoluble fraction of *P. ostreatus* where copper mainly accumulates in the cytosol of *P. ostreatus* (Favero, Costa & Rocco, 1990).

Therefore, the objective for this study is to determine the effect of cadmium and copper on the growth of *P. ostreatus*.

CHAPTER 2

LITERATURE REVIEW

2.1 HEAVY METAL POLLUTION

There are 92 natural occurring elements in the world, among them only 30 metals and metalloids such as arsenic (As), beryllium (Be), cadmium (Cd), lead (Pb) as well as others are toxic to humans. Heavy metals which are widely dispersed in the environment are cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg). They are considered as the most toxic towards animals and humans even at lower concentration (Morais, Costa & Pereira, 2012). Heavy metals able to be exposed to human by air, water, food as well as the commercial products. Not only the above things, workplace can be the reason for heavy metal exposure as some of the industries produced or maybe use heavy metals. Different heavy metals behave differently in human bodies (Heavy metals and your health, 2011).

Heavy metals such as iron (Fe), zinc (Zn) and copper (Cu) can be considered as an important trace element for many of the life organisms. Several food like for example: cereals, legumes and fried potatoes have the heavy metal content for instance: nickel (Ni), copper (Cu) and zinc (Zn) (M & Khan, 2016). Heavy metals normally have high atomic weight and high density if use them to compare with water.

Many human activities for example industries has caused the wide spread of heavy metals into the environment which lead to heavy metal pollution and reduction in human health (Tchounwou, *et al.*, 2012). Besides, some heavy metals can be found in the environment naturally such as rock and soil and dissolve in river and groundwater (Fact sheet: heavy metals and the environment, 2014). Comparing with organic pollutants, heavy metals cannot be easily biodegradable and can remain unchanged in the environment. There are reports shown that heavy metals have the ability to bioaccumulate in humans, animals, plants and even microorganisms and resulted in toxicity in their biological system (Wuana, Okieimen & Imborvungu, 2010).

Heavy metals can lead to various types of pollution such as water pollution, soils pollution and air pollution (Sun, 2012). Metabolic functions able to be disrupted by heavy metals in either two ways, first, they are able to be bioaccumulated in the body and therefore

the glands and the vital organs function are disrupted by the heavy metals. Second, they have the ability to displace the important nutritional minerals and thereby hindering the biological function of the living organisms (Singh, *et al.*, 2011).

2.2 CADMIUM TOXICITY

In periodic table, it belongs to period 5 and d-block (AZoM, 2013). Cadmium (Cd) is a silvery-white, soft and ductile metal which is able cut by a knife. Similar with mercury and zinc, when exposed to air, Cd will tarnish. It has high solubility in acids, low melting temperature, higher corrosion resistance, rapid ion exchange activity, high electrical and thermal conductivity which render it suitable to be fixed into alloys, batteries and for electroplating (Cadmium compounds, 2016). Cadmium can be released into the environment atmosphere from either natural or anthropogenic sources. The main natural source for Cd emission is volcanic activities while anthropogenic sources are mining, waste incineration, nickel-cadmium batteries production, fossil fuel combustion and cement manufacturing (Cadmium, 1998).

Cadmium can interference with essential elements such as by interacting with zinc, magnesium, iron and manganese and resulted in their secondary deficit, disrupting metabolism, causing changes of function and morphology in many organs. Besides, Cd also able to disrupt body signalling and biomolecules and cause alteration of methylation (Sarkar, Ravindran & Krishnamurthy, 2013). Also, Cd can stimulate the production of inflammatory cytokines and downregulate the formation of nitric oxide. It is reported that Cd could increase oxidative stress by increasing lipid peroxidation, depleting protein-bound sulfhydryl groups and depleting glutathione (U.S. Department of Health And Human Services, 2011).

Exposure towards Cd normally occurs through inhalation and ingestion. During inhalation about 5 to 50 % of Cd can enter human body through lungs. Small amount of Cd estimated 1 to 10 % could enter through food and water to the human digestive tract and later into human body. Research shown that if not enough iron and other nutrients in diet, more Cd will be taken up compare to usual. However, Cd could not enter human body through skin (Department of Health And Human Services, 2012). Cigarette smoking can be the main source of cadmium exposure. Research studies shown that the Cd levels in blood and kidney of smoker are much higher compare with non-smoker (Bernhoft, 2013). For non-smoker, major source of Cd exposure are food such as potatoes, cereal products and other vegetables (IARC Working Groups, 2011).