# EFFECTS OF CHROMIUM AND COPPER ON THE GROWTH OF *Monascus purpureus*

MANGAR VISHW

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

July 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 proposal 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.

| | |
|---|---|
| Mangar Vishw | V. Mangar |
| Name | Signature |
| I14006642 | 07/AUG/17 |
| 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 Prof./Assoc. Prof./Dr./Mr./Ms. (and co-supervision of Prof./Assoc. Prof./Dr./Mr./Ms. if any)

Mangar Vishw

Student ID I14006642

DATE/JAN/YEAR 07/AUG/17

Dr. Thong Weng Hing

(SUPERVISOR)

(CO-SUPERVISOR if any)

## ABSTRACT

Heavy metals including copper (Cu) and chromium (Cr) have higher density than water. Recently, with rapid industrialization there has been an exponential increase in their use that has resulted in heavy metal pollution. Unlike other types of pollution, heavy metals are not bio-degradable and therefore, persist in the environment and cause serious detrimental effects on the ecosystem. Bio-accumulation in food chain has dramatically increase their exposure in man. Heavy metals cause serious health problems at even very low levels. For instance, Cr and Cu cause several organ damage, genetic diseases and cancers. Therefore, soil and water contaminated with heavy metals necessitate immediate remediation. Amongst the methods used, bioremediation particularly mycoremediation is both eco-friendly and less costly. Therefore, the identification of these putative fungi for mycoremediation is highly significant and promising. Till date, there is no report on *Monascus purpureus* being studied for bioremedial properties. Therefore, the purpose of this research was to determine the effects of different concentration of Cu and Cr on the growth of *M. purpureus*. Ten different concentrations of Cu and Cr solution (0, 1, 5, 10, 20, 40, 80, 120, 160, 200 mg/mL) were used for the growth of the fungi. Growth of mycelium was monitored for a period of 10 days. Rate of growth was measured by radial growth of *M. purpureus*. The data was statically analyzed by performing one-way ANOVA. *M. purpureus* was able to grow in concentrations of up to 120 mg/L of Cu and Cr. The negative control had the highest average growth rate with 4.63 mm/d. The presence of Cu and Cr in the culture media caused a decrease in growth rate from 4.63 mm/d (negative control) to 0 mm/d in PDA media supplemented with 160 and 200 mg/L (both in Cu and Cr). The lowest growth rate for both Cu and Cr (in the single inoculation) was observed at a concentration of 80 mg/L with 3.10 mm/d and 2.80 mm/d for Cu and Cr respectively. In addition, the lower growth rate in medium containing Cr indicated that Cr might be more toxic. The highest growth rate in Cu containing medium was 4.60 mm/d at concentration of 120 mg/L whereas that of Cr was 4.0 mm/d at a concentration of 10 mg/L. In the double inoculation, the highest growth rate obtained in both Cu and Cr containing media was 3.8 mm/d at the same concentration of 10 mg/L. The highest concentration of Cu and Cr that sustain growth was 120 mg/L and that inhibited growth was 160 and 200 mg/L. This study was done with a view to investigate if the fungi can be a putative bioremediator of heavy metal. This is because for the fungi to be able to remediate those heavy metal it should be able to survive in the presence of these heavy metal. As *M. purpureus* grew on concentrations of Cr and Cu of up to 120 mg/L, it could

play an important role in bioremediation. Therefore, further research need to be done to determine its potential role in mycoremediation of Cu, Cr and other heavy metals.

## TABLE OF CONTENT

## LIST OF TABLES

## LIST OF FIGURES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| APA | American Psychological Association |
| cm | centimetre |
| e.g. | example |
| ed. | editor |
| etc. | et cetera (and so forth) |
| i.e | that is |
| mm | millimetre |
| ml | millilitre |
| p. | page |
| pp. | pages |
| °C | degree Celsius |
| Vol. | volume |
| mm/d | millimeter per day |

# CHAPTER 1

# INTRODUCTION

Environmental pollution is a global issue. With rapid industrialization and increasing use of heavy metals and synthetic xenobiotics, environmental pollution has become a priority concern in many countries (Liu, Su, Zhang, Li & Pei, 2014). Heavy metals that cause pollution include copper (Cu), lead (Pb), zinc (Zn), chromium (Cr), iron (Fe) cobalt (Co), mercury (Hg), cadmium (Cd), arsenic (As) and nickel (Ni). Heavy metal pollution causes serious detrimental effects on the ecosystem as heavy metals not only pollute the soil but also mix with water and get circulated into the food chains (Randhawa, Ahmad, Anjum, Asghar & Sajid, 2014). Water polluted by heavy metals is extremely carcinogenic and toxic even at quite low concentrations, for instance, Cr as low as 50 μg/L can cause toxicity (Tchounwou, Yedjou, Patlolla & Sutton, 2012). Bio-accumulation of heavy metals such as Cu, Cr, Fe and Zn occurs in the gills and livers of fish found in polluted waters (Paulino, Benze, Sadauskas-Henrique, Sakuragui, Fernandes & Fernandes, 2014). Consuming these fish or food contaminated by heavy metals in turns affects the health of consumers (Hussien & Nosir, 2017). Heavy metals such as Cd and Pb cause renal cancer (Sá, Semedob & Cunhac, 2016). Soil polluted by heavy metals is a pressing issue too as it affects both plants and animals (Aminiyan, Aminiyan, Mousavi & Heydariyan, 2016). The study of Sethy and Ghosh (2013) shows that the germination of the seeds of *Brassica Nigra* were delayed in the presence of heavy metal. Similarly, another study revealed that heavy metals namely Pb, Cd, Ni, Cr, Cu, and Zn released from motor vehicles fuel were toxic to plants (Özel, Özel & Varol, 2015).

It is very important to remediate heavy metal contaminated soil and water as heavy metals are not only toxic and cause hazardous ecological effects but most essentially, they are not biodegradable and persist in the environment (Mahmood, Malik & Hussain, 2010; Juel, Chowdhury & Ahmed, 2016). Various means, such as chemical precipitation and oxidation are used to remove heavy metals from the environment (Bazrafshan, Mohammadi, Ansari-Moghaddam & Mahvi, 2015). Bioremediation which is the use of biological means (Wood, Liu, Tang & Franks 2016) is currently the cheapest and least harmful method of getting rid of heavy metals from the environment (Dzionek, Wojcieszyńska & Guzik, 2016). Therefore, bioremediation has a promising role in both environmental safety and public health (Dzionek et al., 2016). Some bacteria, fungi and green plants have been reported to have heavy metal remedial potentials and hence are being used for bioremediation. Mycoremediation, a process of bioremediation using fungi is gaining much attention due to its cost-effectiveness (Chaturvedi et al., 2015).

Fungi have a species-specific affinity for heavy metals in soil, where they sustain life by adsorbing these heavy metals. As a result, they greatly reduce the impact of heavy metals in the environment (Siddiquee et al., 2015). There are numerous species of fungi such as such as *Aspergillus* species and white-rot fungi species that could also play a vital role as a bioaccumulator of metals and could serve to remove heavy metals from polluted environment. Therefore, the application of mycoremediation technologies and the identification of more putative fungi for mycoremediation is highly significant and promising. The fungi *Monascus purpureus* (*M. purpureus*) also known as 'brown button mushroom' has been shown to have several medical uses (Chang, Chuang, Lee & Huang, 2016). However, no study has investigated its potential abilities on heavy metal remediation. Therefore, the aim of this study is to determine the effects of different concentrations of chromium and copper on the growth of *M. purpureus*.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 HEAVY METAL POLLUTION

Heavy metals naturally occur in the environment in very minimal quantities. However, due to human activities there is an increased in concentrations of these metals in the environment causing heavy metal pollution (Effendi, Kawaroeb, Mursalina, & Lestaria, 2016). As a result, heavy metals including, Arsenic (As), copper (Cu), cobalt (Co) and chromium (Cr) accumulate in the soil, water, air and plants. Heavy metals are characterized by their density (denser than water by five times), atomic number and chemical properties. Heavy metals, unlike organic waste, are not biodegradable, hence stay forever in the environment causing water, air and soil pollution (Juel et al., 2016). Özel et al., (2015) showed that vehicles released heavy metals such as Cr, Cu, Pb, Ni, and Zn that were toxic to plants. Lead (Pb) originated from the tetra-ethyl-lead added into the petrol, Ni from diesel fuel and motor oils, whereas Cd and Zn from automobile tires and oils.

Heavy metal ions cause destabilization of cell structures including DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) as they have strong electrostatic attraction and high affinities to attach to the same locations where essential metal ions normally bind; thus, they induce replication defects, hereditary genetic disorders, and cancers (Yuan, Yang & Li, 2016; Beyersmann & Hartwig, 2008; Jaishankar, Tseten, Anbalagan, Mathew & Beeregowda, 2014). Chromium and Cu have also been shown to cause cancer (Effendi et al., 2016). Similarly, As and Cd are toxic heavy metals classified as human carcinogens - Group 1 (International Agency for Research on Cancer, 2017). Cd has been shown to induce cytotoxic effects at concentrations 0.1 to 10 mM (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Likewise, Morcillo, Esteban and Cuesta (2016) found that Cd caused oxidative stress, leading to apoptosis in fibroblast SAF-1 cells from the marine gilthead seabream. Lead too has severe ill-effects on human health whereby exposure to level higher than 5 µg/dl can cause severe kidneys and brain damage and ultimately cause death (Martin & Griswold, 2009). Likewise, mercury also causes permanent brain and kidney damage, and affect developing fetuses (Bose-O'Reilly, McCarty, Steckling & Lettmeier, 2010).

## 2.2 CHROMIUM (Cr)

Chromium, even though naturally present in the environment is a toxic heavy metal. Hazards due to environmental Cr contamination depend on its oxidation state and solubility. Chromium exhibits various oxidation states, ranging from 0 to +6, which direct its chemical reactivity with chromium (III) being the most stable form of the element. Cr, is commonly used, in cement, paints, construction industries, and leather tanning industries (Homam, Haile & Washe, 2016). Developing countries have fewer laws and structure supports to protect their environment. Thus, as argued by Singh and Rajamani (2003), developing countries are the ideal location for companies dealing with leather tanning, as they allow the companies to spend less capital to deal with Cr containing wastewater. The effects of Cr depend highly on the route of exposure and its chemical form. Inhalation of chromium lead to damage in the respiratory system, whereas, dermal exposure lead to inflammation (Chen, Lee, Yeh, Wang & Wang, 2016). Moreover, intake of large amounts such as 100 µg/L of Cr (VI) has been found to cause kidney and liver damage (Collins et al., 2010) and overexpression of p53 cancer-causing gene (Elhosary, Maklad, Soliman, El-Ashmawy & Oreby 2014; Banu et al., 2011; Hill et al., 2008). Chromium has also been proven to cause damage to DNA (Tchounwou et al., 2012).

The study by Thilini, Jagath and Gunaratn (2015) showed that the level of heavy metals including Cr was higher in vegetables on the market than those collected from the production sites implying that heavy metal can be deposited on vegetables from polluted atmosphere during transport and marketing. This study also revealed that different processing techniques including any form of cooking had no effects on the average levels (mg/kg) of Cr in the studied vegetable. As washing or heat processing cannot remove heavy metals from contaminated food it is imperative to apply other means to remove these dangerous particles from the atmosphere. As the acute oral toxicity range is between 1900 and 3300 µg/kg, it can be deduced that people eating Cr contaminated food will have serious health problems (Coelho et al., 2015). It was reported that cow feeding on contaminated nutrients eventually result in the milk being contaminated by 0.284±0.021 mg/L of Cr (Bilgücü, Kaptan, Palabiyik & Oksuz, 2016). Furthermore, milk being the second most consumed liquid in the world, there is no doubt this matter will worsen if