DETERMINATION ON THE PRESENCE OF AFLATOXINS AND OCHRATOXIN A OF FUNGI USED IN BIOREMEDIATION

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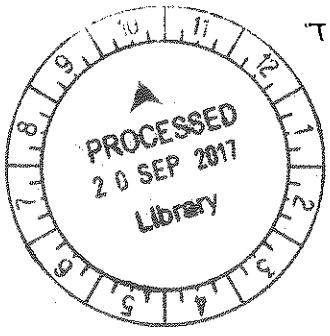
DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF BIOTECHNOLOGY (HONOURS)



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ABSTARCT

Pollution was largely caused by the anthropogenic discharge of harmful chemicals which resulted in heavy metal contamination. There are many ways to remove these pollutants but may be expensive and not environmental friendly. Mycoremediation was a preferable method to remove these pollutants because it was cheap, environmental-friendly and possible to completely degrade pollutants. In mycoremediation, fungi were used as a remediation tool to remove metals. However, fungi produced biologically active compound which was toxic to animals and plants called mycotoxins. The objective of this study was to determine the safety usage of several fungi species used in mycoremediation. Nine different species of fungi were tested for the presence of ochratoxin A and aflatoxins which were two common mycotoxins using thin layer chromatography (TLC). The TLC plate was viewed under UV and compare with the standards R_f values of the mycotoxins. It was found that all the fungi species did not produced aflatoxins. *Trichoderma sp.* was found to produce ochratoxins A but not in others fungi species. Therefore, *Trichoderma sp.* was shown to be a poor candidate for mycoremediation as it was not safe and the rest of the fungi isolates were potential candidates for mycoremediation.

Keywords: Pollution, Mycoremediation, TLC, Aflatoxins, Ochratoxins A

TABLE OF CONTENT

		Page
NON-PLAGIARISM DECLARATION		**i**
DECLARATION		**ii**
ACKNOWLEDGEMENTS		**iii**
ABSTRACT		**iv**
TABLE OF CONTENT		**v**
LIST OF TABLES		**vii**
LIST OF FIGURES		**viii**
LIST OF ABBREVIATIONS		**ix**
CHAPTER		
1	**INTRODUCTION**	**1**
2	**LITERATURE REVIEW**	**3**
2.1	Pollution	3
2.1.1	Source and cause of pollution	3
2.1.2	Effects of pollution	4
2.2	Mycoremediation	4
2.2.1	Fungi as a bioremediation tool	4
2.2.2	Mechanisms of mycoremediation	5
2.3	2.3.1 Fungi	6
2.4	Mycotoxins	7
2.4.1	Aflatoxins	8
2.4.2	Ochratoxins A	9
3	**MATERIALS AND METHODS**	**10**
3.1	Fungi cultivation	10
3.2	PDA media preparation	10
3.2.1	Plating of PDA	10
3.2.2	Subculture of fungi into PDA medium	10
3.3	PDB medium preparation	11
3.3.1	Pouring of PDB medium	11
3.3.2	Subculture of fungi into PDB medium	11
3.4	Mycotoxins study	12
3.4.1	Extraction of mycotoxins	12
3.5	Solid phase (SPE) clean up	12
3.5.1	Conditioning the cartridge	13

3.5.2 Washing the cartridge 13
3.5.3 Eluting the cartridge 14
3.6 Mycotoxins detection by thin layer chromatography (TLC) 14
3.6.1 Aflatoxins detection 14
3.6.2 Spottings of aflatoxins 15
3.6.3 Ochratoxins detection & spotting 16
3.7 Reference R_f value 17

4 RESULTS 18
4.1 Fungal isolates in PDA and PDB 18
4.2 Mycotoxins Analysis 18
4.2.1 Ochratoxin A detection 19
4.2.2 Aflatoxins detection 20

5 DISCUSSION 25
5.1 Fungal isolates 25
5.2 Mycotoxins detection 25
5.2.1 Ochratoxin A identifiifcation 26
5.2.2 Aflatoxins identification 27
5.3 Safety usage of fungi for bioremediation 27

6 CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH 29

7 REFERENCES 30

LIST OF TABLES

Table		Page
3.1	Developing solvent of aflatoxins	15
3.2	Solutions of dye mixture to spot aflatoxins	15
3.3	Developing solvent of ochratoxin A	16
3.4	Solutions of dye mixture to spot ochratoxin A	16
3.5	Standard R_f values of Aflatoxins	17
3.6	Standard R_f values of Ochratoxin A	17
4.1	R_f values of spots in replicates travelled on TLC for ochratoxin A detection	19
4.2	R_f values of spots in replicates travelled on TLC for aflatoxins detection	20

LIST OF FIGURES

Figures		Page
2.1	Mycelial network (left) and fruiting body (right)	6
2.2	Filamentous fungi	6
3.1	Sub culturing of fungi in PDB medium	11
3.2	Washing of cartridge	13
3.3	Eluting of samples in cartridge.	14
3.4	Preparation of TLC plate (a) and running of TLC plate (b). Heating of TLC plate to develop fluorescent spots (c).	15
4.1	Replicate 1 of TLC under UV illuminator of ochratoxin A detection	22
4.2	Replicate 2 of TLC under UV illuminator of ochratoxin A detection	22
4.3	Replicate 3 of TLC under UV illuminator of ochratoxin A detection	22
4.4	Replicate 1 of TLC under UV illuminator of aflatoxins detection	24
4.5	Replicate 2 of TLC under UV illuminator of aflatoxins detection	24
4.6	Replicate 3 of TLC under UV illuminator of aflatoxins detection	24

LIST OF ABBREVIATIONS

Mg	Milligram
L	Litre
OT	Ochratoxins
AF	Aflatoxins
ZEN	Zearalenone
F	Fumonisins
mL	Millilitre
g	Gram
°C	Degree Celsius
Kg	Kilogram
Min	Minutes
LAF	Laminar Air Flow
%	Percentage
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
TLC	Thin Layer Chromatography
UV	Ultraviolet
cm	Centimetre
R_f	Retention Factor

CHAPTER 1

INTRODUCTION

The environment has been polluted with synthetic organic compounds and it has become a major problem worldwide. Since modernization and development in the environment, there has been a considerable increased in the amount of pollution. Therefore, pollution starts to reach a peak because these pollutants are difficult to be metabolized and be broken down into non-toxic forms and will leave behind a long lasting effects on the environment (Dixit et al., 2015).

It becomes a requirement to promote the process of eliminating pollutants and wastes from the environment. Nonetheless, due to the lack of efficient solution, a rapid, practical ecologically procedure of clean-up is greatly required. Modern techniques remove pollutants using tools, chemicals and equipment while when the concentrations of pollutants are less than 100 mg/L, it becomes unproductive. It is also economically infeasible because large area is infected. Alternately, environment friendly using bioremediation methods using plants, fungi or microorganisms for pollutants removal may be an option (Dixit et al., 2015).

The ability of fungi to transform a large variety of hazardous chemical have rises significantly in using them for bioremediation purposes (Sullia, 2008). Fungi has the properties of being a proficient molecular disassembler by breaking down long chain toxins into simpler molecules. Several fungi also possessed mycelial enzymes which enable them to decompose some of the toughest materials such as petroleum products or estrogen-based pharmaceuticals. It is therefore effective to remove pollutants because these wastes become vulnerable to the degradation of mycelial enzyme. The approach of using fungi for bioremediation is also benefitting because it helps to set up stage for other organisms such as plant or bacteria to participate in the bioremediation process (Paul Stamets & Crimethinc, 2005).

Unfortunately, fungi produce a secondary metabolite called mycotoxin. It is toxic and a contaminant found. In agricultural and foods materials, it caused foods to become unsafe to be consumed by humans and animals (Rahmani, Jinap, Soleimany 2009). One of the known effects of exposure to mycotoxins is it causes liver toxicity and cancer (Wu, 2015). There are thousands of mycotoxins in existence but only a few are considered as hazards ("Determination of Mycotoxin Residues by LC - MS/MS Featuring Two Alternate Sample Extraction Procedures," 2014).

Therefore, analysis of mycotoxins produced by fungi is essential to detect their presence in the environment to ensure that they do not cause harm when it is used for mycoremediation.

The aims of this experiment were

1. To detect the presence of aflatoxins and ochratoxins A.
2. To determine the safety usage of several species of fungi in bioremediation using TLC.

CHAPTER 2

LITERATURE REVIEW

2.1 POLLUTION

Pollution is defined as a process where harmful pollutants are introduced into the natural environment and causes adverse effects and changes. In the early centuries, environmental pollution has already existed but is not of a concern until it started to be of significance in the 19th century because over the recent years, despite extensive efforts that have been carry out to clean up the pollutants, pollution still remains as a major problem ("Causes and Effects of Environmental Pollution - Conserve Energy Future," n.d.).

2.1.1 Source and Cause of Pollution

Since the global industrial revolution started, the problem of pollution is of no doubt will reached its greatest and serious proportion in the densely populated developing urban-industrial centers (Ahmad Khan MBBS & Mujahid Ghouri, 2011). The most common pollution happening in the developing world are largely caused by unsustainable anthropogenic discharge of industrial emission, inefficient waste management, contaminated river leading to poor water sanitation, exposures to air pollution from gas emitted through fuels and at the same time impulsive applications of chemical fertilizers containing heavy load of metals which will result in hydrocarbon such as petroleum and heavy metal contamination (Ali, Ali, Islam, & Rahman, 2016).

Heavy metals mean a group of metals and metalloids having a density greater than 4 g/cm^3 (Khan, Goel & Musarrat, 2011). Heavy metals are recognized worldwide as widespread contaminants found in the ecosystem usually in water sources and terrestrial. Although heavy metals are considered as contaminants, they are occurring naturally in the crust of the earth and are found in rocks, soils, waters and sediments (Wuana, Okieimen, Wuana, & Okieimen, 2011).

When heavy metals concentration increased many times greater than natural the sources in the environment caused by careless human actions, it has a tendency of being retained and persist in the environment because it is difficult to degrade and destroyed (Ahmad Khan MBBS & Mujahid Ghouri, 2011). Presence of heavy metals as waste due to their uses in modern society is an ever-growing concern globally and has become a threat to the environment (Mohammed, Kapri & Goel, 2011).

On the other hand, hydrocarbon pollution caused by sources such as petroleum and crude oil are also of great concern because they are toxic to all forms of life. Environmental contamination with hydrocarbon is relatively common because of its relatively widespread uses and is associated with its disposal operations and accidental spills in seas (Abha & Swaranjit, 2012).

2.1.2 Effects of Pollution

When pollutions were introduced as different forms of waste materials into the environment, there will be negative impacts to the ecosystem. Its impacts include global warming, lung diseases and contaminated seafood. Water which were contaminated by chemicals such as lead and hydrocarbon can cause hormonal and reproductive problems, damage to the nervous system, liver and kidney damage and cancer. Being exposed to mercury causes Parkinson's disease, Alzheimer's, heart disease and death ("How Does Pollution Affect Humans? - The World Counts," 2014).

When a susceptible individual is exposed to the high amount of circulation of these toxic heavy metals for a long period of time, it will also triggered health risk associated with it and developed detectable symptoms (Briggs, 2003). For example, acute or chronic damage to the nervous system is associated to long term exposure to lead while long term exposure to cadmium causes renal dysfunction and obstructive lung disease. Diseases such as lung cancer and damage to human's respiratory systems have also been linked to cadmium exposure (Khan et al., 2011).

Therefore, there is a need to quickly address the problem associated with environmental pollution. Effective and sustainable methods using environmental

friendly and cost effective measures can be taken e.g. microorganisms and fungi (Ahmad Khan MBBS & Mujahid Ghouri, 2011).

2.2 MYCOREMEDIATION

2.2.1 Fungi as mycoremediation tool

Mycoremediation is a process of remediation using fungi to break down pollutants such as heavy metals in the environment (Kulshreshtha, Mathur & Bhatnagar, 2014). They are opportunistic and respond to their environment quickly ("Introduction to Mycoremediation - Breaking down complex, toxic molecules and pathogenic organisms | Mushroom Mountain," n.d.). Both microorganisms such as bacteria and fungi are both used for degrading pollutants but fungi are preferable because the ability of bacteria to degrade is limited. The energy of bacteria is low, have low bioavailability and hence cannot break down rare elements (Daphne, Gnanasalomi, Jebapriya, & Gnanadoss, 2013). Fungi innate ability to degrade or to deteriorate a wide range of complex hydrocarbons and various toxic and hazardous compounds while disassembling the long chained toxins into less toxic substrates has earned its role as a remediator in bioremediation (Singh & Harbhajan, 2013).

2.2.2 Mechanisms of mycoremediation

The main mechanisms of mycoremediation of fungi of degrading substances is performed by placing the mycelial networks over toxic area (Hansen James, 2012). There are powerful extracellular enzymes and acid being secreted by the mycelium as shown in figure 2.1 that also digests lignin and cellulose, the two essential building blocks of plant fibre like wood. These enzymes can therefore break down organic compound, like toxins which have similar bonds found in wood (Daphne et al., 2013). Fungi will also remove pollutants by transporting them into their fruit-bodies (Hansen James, 2012). In the choice of fungi, filamentous fungi are more advantageous because essential factors (pollutants, nutrients and water) are required in order for degradation to start and these fungi are able to tanslocate the essential factors through different parts of the mycelium (Daphne et al., 2013).