SCREENING OF POTENTIAL FUNGI FROM THE POLLUTED SOIL FOR COPPER BIOREMEDIATION

CHRISTOPHER LEERAJ A/L SOLOMON DEVARAJ

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FACULTY OF SCIENCE, TEHCNOLOGY,
ENGINEERING AND MATHEMATICS
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ABSTRACT

Microorganisms play a major role in copper remediation from the polluted soil. To control metal pollution, the bioremediation method by utilizing fungi can be applied. The objective of this study was to screen out the fungi potential for bioremediation of copper. The soil sample was collected from a steel factory called Amsteel mill in Klang. The fungus was screened out on Rose Bengal agar and cultured on potato dextrose agar to obtain pure culture for toxicity test. Fungi were subjected to toxicity test using copper sulphate concentration up to 300 ppm. Fungi were identified macroscopically by using pictures and microscopically by means of slide culture technique alongside relevant websites. The result showed that up to a total of 4 potential fungi species based on its growth rate, which were *Trichophyton verrucosum* being the best followed by *Aspergillus niger, Candida spp.* and *Aspergillus nidulans* were able to tolerate and grow best in copper and hence making them potential candidates as bioremediation agents. A total of 6 non potential fungi were discovered which were *Microsporum cookei, Chrysosporium spp., Trichosporon asteroides, Beauveria spp., Paecilomyces spp.* and *Wangiella dermatidis.*

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LIST OF ABBREVIATIONS

A.nidulans	*Aspergillus nidulans*
A.niger	*Aspergillus niger*
BAM	Bacteriological Analytical Manual
CFU	colony forming unit
°C	degrees Celsius
g	gram
L	litre
mg	milligram
MIC	minimum inhibitory concentration
M.cookie	*Microsporum cookie*
NaCl	Sodium chloride
PDA	potato dextrose agar
ppm	parts per million
PDB	potato dextrose broth
RBA	rose bengal agar
T. mentagrophytes	*Trichophyton mentagrophytes*
T. verrucosum	*Trichophyton verrucosum*
T. asteroids	*Trichosporon asteroids*
W.dermatitidis	*Wangiella dermatitidis*
WHO	World Health Organiztion
YES	yeast extract/ sucrose

1.0 CHAPTER 1

INTRODUCTION

Bioremediation is generally known as the utilization of organisms in the treatment process of polluted soil. Soil pollution could be from many sources such as heavy metals which are toxic metal pollutants. Many of these toxic metal pollutants originate from waste products of metallurgical and industrial processes. Heavy metals such as lead and copper are released into the environment and hav e been found to be increasing continuously over the years from technological development and industrial activities. In the industrial context, these metals have to be reduced upon disposal to meet certain legislative standards. According to World Health Organization (WHO), copper is one of the most major and most immediate concerns. This is because copper is a toxic metal that affects water sources as the effluent from metal finishing processes could contain up to a total of 10 mg/L of copper. Utilization of methods such as reverse osmosis and chemical precipitation, has shown results such as incomplete metal removal proving that these methods were ineffective. In addition, it also generates toxic sludge which requires proper disposal and having high energy requirements. These methods are also considered to be uneconomical compared to bioremediation.

One way of bioremediating soil is through the use of fungi in a process called mycoremediation. Fungi are generally utilized as they are a versatile biosorption group which are able to tolerate, grow and survive under extreme conditions such as high temperature, high pH and high metal concentration. Biosorption is a bioremediation process which involves the utilization of microbes to firstly detoxify environmental contaminants and then control the contaminants. The biosorption process has been utilized recently to clean up polluted sites. In the bioremediation industries, filamentous fungi such as *Aspergillus* and *Penicillium* are fungi which have been widely utilized for decades. Fungi have been employed in the remediation of wastes and waste water. Over the years there has been growing research in the utilization of biomass from fungi for

means of bioremediation which has been producing a broad and promising range of results.

In this study, the potential bioremediation fungi can be found in polluted soil sample near factory areas. With bioremediation, environmental pollution by copper should be considered nothing but a misplaced resource as it can be recovered through means of bioremediation and then utilized for the processing of many commercial products and industrial materials. Through effective methods such as metal recovery and biomass production, the heavy metals can be removed from the source utilizing the fungi in order to reducing the toxic effects these metals exerted onto the environment.

The objectives of this research were as followed

1) To screen for potential fungi for copper bioremediation in different concentrations of copper toxicity test.
2) To identify the potential fungi for copper bioremediation through macroscopic and microscopic features.

2.0 CHAPTER 2

LITERATURE REVIEW

2.1 COPPER AS A HEAVY METAL

Copper contamination has evolved to become a major issue in the twenty first century due to its continuous release into the environment. The sources were mainly from effluents produced by metal processing and mining (Savvaidis et al., 2003). Based on Figure 2.1 copper production is seen to be originated from factories which undergo mining and milling through pyrometallurgical processing. This greatly impacts the land quality (United States Geological Survey, 2001). The rapid development of industries such as agriculture as well as natural activities, copper has been massively discharged into soil (Navarro et al., 2008). Copper present in contaminated soil just like any other heavy metal, can pose a threat to human health when it enters into the food chain (Arora et al., 2008). From the total of 1,647 hazardous waste sites that have been proposed for inclusion on the Environmental Protection Agency National Priorities List, a total of 906 of them were found to contain copper (Hazdat, 2004).

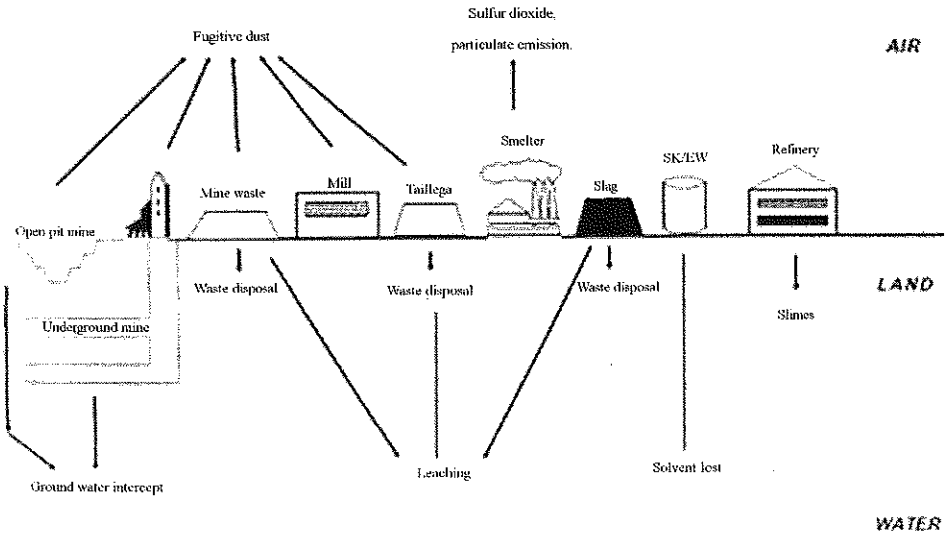

Figure 2.1 Environmental impacts of copper production.

Main sources of copper ions in the environment originate from metallurgical and chemical manufacturing (Cortes et al., 2003). An example of it is the smelting process which releases a large amount of copper. This will give a negative impact on human health. The source of pollution are air emissions and wastes process from smelting such as slag as shown in Figure 2.2. In older copper smelters, air emissions contained elevated levels of copper which dispersed by air and contaminate nearby soil and destroying vegetation. For industrial activities in the United States of America, which involve copper production were subjected to extensive environmental regulation which includes materials handling and disposal practices (U.S. Geological Survey, 2001).

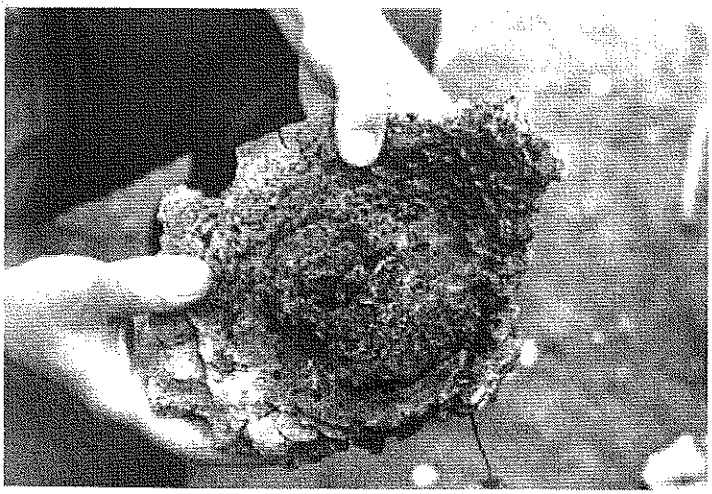

Figure 2.2 A piece of copper slag from a smelting furnace.

2.2 POTENTIAL OF FUNGI FOR BIOREMEDIATION

2.2.1 General Characteristics of Potential Fungi for Bioremediation

Fungi are known to be unique organisms for bioremediation due to their physiological, morphological, and genetic features. They are also ubiquitous, able to colonize all matrices hence making them suitable for bioremediation (Anastasi et al., 2013). Fungi also thrive in soil and this further strengthens its potential for bioremediation of many heavy metals in soil. Metal tolerant fungi are utilized in bioremediation due to factors such as eco-friendliness and high efficiency. Recent advances have been made to fully understand the interaction between metal and microbe due to their application in metal detoxification (Rajendran et al., 2002). Some fungi, such as *Aspergillus niger,* the pathogenicity is reduced causing less harm to humans and animals; thus this prevents the unwanted spread of disease which is another beneficial factor for bioremediation (Anastasi et al., 2013).

2.2.2 Filamentous Fungi for Bioremediation

Filamentous fungi is known as any group of saprobic fungi causing a cottony growth on organic substances or also known as the deposit produced by fungi. Filamentous fungi are well known in fermentation industry for its production of diverse metabolites such as enzymes and antibiotics. In addition to it, in the field of bioremediation fungi shows great affinity for metals ions in comparison to other microbes. In terms of physicochemical and biological mechanisms, they can be utilized to accumulate metals such as copper from the external environment (Cabuk et al., 2004).

A group of filamentous fungi belonging to the *Zygomycetes* group are few of these potential fungi for bioremediation (Madigan et al., 2000). They also contain large amounts of polymer of N-acetyl, chitosan and chitin, and deacetylated glucose-amine on their cell wall that provides large quantities of potential binding sites for metals. These potential binding sites includes free hydroxyl groups, carboxyl and amine. The presence

of an amine group which contains nitrogen atoms as well as a hydroxyl group containing oxygen atom, it enables the binding of copper (Das & Guha, 2007). However, there were some issues such as the electro-negativity of oxygen being higher than nitrogen which leads to a lone pair of electrons being donated from the nitrogen. These lone pair of electron donated from the nitrogen can be more facile compared to the oxygen atom in the formation of metal complex (Das & Guha, 2007).

2.3 BIOREMEDIATION PROCESS AND BIOSORPTION CAPABILITIES OF FUNGI

2.3.1 Bioremediation Process

Mycoremediation is a form of bioremediation and is generally known as the process utilizing fungi to return soil and wastewater contaminated by pollutants to a less contaminated state. Mycoremediation works by channeling the heavy metals from the soil to the fruiting bodies of the fungi which contain spores, for removal (Okhuoya, 2011). One of the primary roles of the fungi in the ecosystem is decomposition which is carried out by the mycelium. Hence, determining the right fungal species to target a specific pollutant is the key to mycoremediation. Mycofiltration is also a similar process to mycoremediation which uses fungal mycelia to filter toxic waste and microorganisms from water in soil.

The future of mycoremediation lies in Mycological Response Teams (Stamets, 2004). Basically the Mycological Response Teams consist of knowledgeable and trained people who would set up centers that would use mycoremediation techniques for the purpose of recycling and rebuilding healthy soil in the area. This all began by spreading awareness through information of the benefits of mycoremediation (Stamets, 2004)