SCREENING AND IDENTIFICATION OF POTENTIAL FUNGI FROM POLLUTED SOIL FOR THE BIOREMEDIATION OF COPPER AND LEAD

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ABSTRACT

Heavy metal pollution is one of the most prominent environmental problems faced by countries all over the world, leading to extensive research in how to eliminate this problem. Out of several available methods for environmental detoxification, bioremediation is gaining popularity due its multi-advantageous process. The use of fungi is one of the best ways to remediate heavy metals in soil. This study focuses on the screening and isolation of fungi from polluted soil for the bioremediation of copper and lead, using rose Bengal agar and potato dextrose agar. Fourteen different fungal species were identified using microscopic and macroscopic analysis and were subsequently subjected to heavy metal toxicity testing using lead and copper. Among all isolated species, *Trichoderma sp., F. oxysporum, P.citri and Fusarium sp. 1* were found to be good candidates for the bioremediation of both lead and copper, while *Mucor sp.* showed promising results with lead and *Fusarium sp. 2* showed good results with copper.

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LIST OF ABBREVIATIONS

CFU	Colony Forming Unit
°C	degrees Celsius
G	Gram
L	Litre
Mg	milligram
PCR	Polymerase chain reaction
NaCl	Sodium chloride
PDA	Potato dextrose agar
ppm	Parts per million
RBA	Rose bengal agar
µL	Microliter
SPSS	Statistical Package for the Social Sciences
Pb	Lead
Cu	Copper

CHAPTER 1

INTRODUCTION

Heavy metal pollution is the excessive release of heavy metals into the environment and mainly results from human activities (Iram, Uzma, Sadia & Talat, 2013), as heavy metals have several applications in a variety of fields, such as industrial, medical and technological to name a few (Tchounwou et al., 2012). The major source of soil pollution comes in the form of point sources (He, Yang & Stoffella, 2005) -from mining, smelting and other metal-based activities (Tchounwou et al., 2012). Soil pollution by heavy metals poses a great threat due to the possibility of these metals persistently circulating in the environment (Volesky & Holan, 1995) and finally entering food chains (Iram et al., 2013a). The accumulation of these heavy metals can cause extensive damage to the body (Abdel Salam, Reiad & ElShafei, 2011), resulting in several detrimental human conditions and diseases (Tchounwou et al., 2012), as many of these heavy metals are not just toxic, but also exhibit carcinogenic and mutagenic effects (Dixit et al., 2015). Despite their adverse effects in high concentrations, when found in lower concentrations, some heavy metals, such as copper and zinc, function as essential micronutrients in several organisms (Ivanov, 2008).

The dangers of heavy metal pollution are the reason why clean-up is necessary. There are several physical-chemical methods that are currently available, like floatation, ion exchange (Varma, Singh & Sahu, 2013), electrochemical deposition and chemical precipitation (Dixit et al., 2015; Barakat, 2011, Varma et al., 2013). However, there are several disadvantages to these methods, the most important one being that they are expensive processes (Dixit et al., 2015) that utilize large amounts of chemicals that in turn may contribute to pollution levels (Barakat, 2011). Another disadvantage is that many of these methods are ineffective at concentrations below 100mg/L (Dixit et al., 2015). For these reasons, as mentioned by Dixit et al. (2015) and Iram et al. (2013a), bioremediation has become one of the best alternatives for environmental clean-up.

Bioremediation is the use of biological organisms to clean up contaminants from soil and water environments (EPA, 2012). Research into mycoremediation, or the use of fungi for bioremediation (Le, 2013), became popular in recent decades, due to fungi's biosorption capabilities, high adaptability to extreme conditions and the inexpensiveness of the process (Çabuk, Ilhan, Filik, & Çaliskan, 2004). This research focused on isolating fungi for the bioremediation of one essential metal, copper, and one nonessential metal, lead. Such potential fungi can be isolated from soil in the vicinities of factories and industrial areas, allowing researchers to obtain fungi with greater affinity towards specific heavy metals. These remediated metals can then be recovered and re-used in industrial processes, creating a sustainable cycle and enhancing the importance of metal recovery through bioremediation.

The objective of this research was:

1. To isolate and screen for potential fungi in polluted soil for the bioremediation of copper and lead through toxicity testing.
2. To identify the isolated fungi using microscopic and macroscopic features.

CHAPTER 2

LITERATURE REVIEW

2.1 HEAVY METALS

2.1.1 Copper

Copper in its purest natural form is found as a reddish-brown metal, exhibiting typical heavy metal characteristics such as having a high melting point, malleable properties and it is able to conduct electricity, while also having good corrosion resistance properties (SEPA, 2015a). Due to these characteristics and properties, copper is widely used in a range of industries and fields, including but not limited to, electronics, construction, agriculture and etc. (SEPA, 2015a).

Excessive copper release into the environment mainly results from anthropogenic activities such as metal processing, mining (Savvaidis, Hughes & Poole, 2003) smelting and agriculture (Suciu et al., 2008), as well as from sewage treatment processes (SEPA, 2015a). A small percentage of copper pollution is also a result of natural release through rock weathering and atmospheric deposition (Nirel & Pasquini, 2010). The majority of copper pollution in both soil and water can be traced back to point sources like factories dealing with metallurgical and chemical manufacture (Navarro et al., 2008).

Copper pollution is detrimental to both the environment and human health (Lente et al., 2014). Excessive copper in soil can disrupt nutrient cycles carried out by microorganisms thus inhibiting the recycling of essential nutrients like nitrogen and phosphorous (SEPA, 2015a), affecting the growth and quality of crops and other vegetation (Lente et al., 2014). Copper cannot be biodegraded, so it accumulates in the environment (Nirel & Pasquini, 2010), entering food chains and leading to various chronic conditions in humans (Lente, 2014).