THE USAGE OF FUNGI IN CADMIUM AND MANGANESE REMEDIATION

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ABSTRACT

Heavy metals are found in the soil naturally, but certain activities such as industrial processes cause an increase in the concentration of heavy metals to amounts that will become toxic to the environment. Pollution with heavy metals such as Cadmium (Cd) and Manganese (Mn) is becoming an extremely serious environmental issue that affects plants, animals and humans. In order of overcoming pollution of Cd and Mn, Bioremediation poses as one of the best techniques to remove Cd and Mn from the environment due to its ease of application and low cost. The aim of this research was to study the potential of different fungal species in bioremediating Cd and Mn. Fungi were grown in Potato Dextrose Agar (PDA) media and then cultured in Potato Dextrose Broth (PDB) with different concentrations of Cd and Mn to test the ability of the fungi to grow and resist the toxicity of the heavy metals. The rate of growth of each fungus was obtained after one week by measuring the dry weight of every fungus on each concentration of toxicity by Cd and Mn respectively. The best strain of fungi was determined based on their ability to grow in the presence of the heavy metals. *A. flavus* showed the significantly highest rate of growth of 0.033667±0.001 g/week at 110 ppm which means that it had the best resistance towards the high Cd toxic conditions. *P. chrysogenum* showed the significantly highest rate of growth of 0.068333±0.010 g/week at 500 ppm of manganese. The fungal species were able to grow with Cd and Mn due to the many mechanisms they employ to uptake heavy metals such as biosorption.

Keywords: bioremediation, cadmium, manganese, fungi.

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LIST OF ABBREVIATIONS

Cd	Cadmium
mg	milligrams
g	gram
mg/kg	milligram per kilogram
L	liter
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
ppm	Par per million
EPA	Environmental Protection Agency
mL	millilitre
mg	milligram
g/week	grams per week
ºC	Degree Celsius

CHAPTER 1

INTRODUCTION

All around the globe, pollution with heavy metals is becoming an extremely serious environmental issue that affects plants, animals and humans (Xie et al., 2016). The heavy metals that concern us due to occupational or residential exposure are a total of 35 metals: arsenic, cadmium, bismuth, silver, thallium, manganese, antimony, tellurium, chromium, nickel, copper, cerium, gold, cobalt, gallium, lead, iron, mercury, uranium, platinum, vanadium, tin, and zinc (Jaishankar, Tseten, Anbalagan, Mathew, & Beeregowda, 2014). Although these metals are found in the soil naturally, certain activities that can be either anthropogenic or geologic such as miming, use of pesticides and fertilizers in agriculture, production of metal products like batteries and disposal of public waste and sewage sludge cause an increase in the concentration of heavy metals to amounts that will become toxic to the environment (Chibuike & Obiora, 2014).

Cadmium is a non-essential heavy metal that can be found naturally in the earth's crust and it has been found to impose a threat to human health when its concentrations crosses a certain threshold of 70 ppm according to the EPA (Grubinger & Ross, 2011). It's a major pollutant of the environment and especially the soil, as it can pollute the soil through industrial activities (Wilson, 1988). It can also enter the food chain easily which calls for the importance of bioremediation to remove it from the soil and maintain a safe environment and a proper human health.

Manganese is another heavy metal that is essential and is emitted into the environment. According to the Environmental Protection Agency (EPA), when the concentration of Mn crosses the threshold of 300 ppm in concentration, it imposes a serious threat to the human health (Horneck, Sullivan, Owen, & Hart, 2011). Mn being an essential heavy metal is very important for human health when consumed in small amounts, so the concentration of Mn in the environment and in the diet must be monitored and controlled in a way that keeps it from causing harm.

One of the techniques that are helpful in cleaning up the environment and ridding it of heavy metals is bioremediation. It is considered to be an innovative technology that shows promising results in the removal of heavy metals from the soil (Girma, 2015). A more specific and more effective type of bioremediation is mycoremediation, and it is the type where the kind of microorganism used in the recovery of heavy metals from the soil is fungi or mushrooms (Purnomo et al, 2013; Kulshreshtha et al, 2013). These fungi have an enzymatic machinery that allows them to be used for the purpose of degrading heavy metals and other types of waste using many processes like biosorption and bioconversion that makes the removal of heavy metals from the soil more effective, faster and much cheaper than any other technique (Kulshreshtha, Mathur, & Bhatnagar, 2014).

Since the pollution by Cd and Mn in the soil has a vast effect, a solution needs to be found in order to prevent these heavy metals from finding their way into the food and insure food safety for the maintenance of human health (Liu et al., 2015). Therefore, in this study, the effect of the heavy metals Cadmium and Manganese were tested against the rate of growth of various species of fungi.

The aim of this research is to determine the remediating ability of several species of potential fungi based on their tolerance to the presence of Cd and Mn at different concentrations.

CHAPTER 2
LITERATURE REVIEW

2.1 HEAVY METALS

Heavy metals are element that are found naturally in the crust of the earth but are called heavy metals due to their toxicity despite their density or mass. They can be classified as either essential or non-essential depending on whether they are nutrients that are essential in many physiological and biochemical functions if in low concentrations. If there wasn't an adequate supply of these essential elements such as manganese, copper, iron, chromium and many others then that would lead to many syndromes or deficiency diseases (WHO, 1996).

Heavy metals will become extremely toxic to humans, living things and the environment when their concentrations crosses a certain threshold that is specific for each metal. They can enter the surrounding environments naturally and through many human activities such as miming, use of pesticides and fertilizers in agriculture, production of metal products like batteries and disposal of public waste and sewage sludge (Chibuike & Obiora, 2014).

2.1.1 Cadmium

Cd is a non-essential heavy metal that is distributed widely in the crust of the earth at a concentration in average of around 0.1 ppm (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). According to the (EPA), Cd has been found to impose a threat to human health when its concentrations crosses a certain threshold of 70 ppm (Grubinger & Ross, 2011). It can also pollute the environment and especially the soil through industrial activities such as the use of Cd compounds in the production of rechargeable nickel cadmium batteries (Wilson, 1988). Health risks and compromise of food safety are caused by the entrance of Cd into the food chain via crops that are grown in the soil with bioaccumulation of Cd (Liu, Qian, Cai, & Yang, 2006). It is considered as a carcinogen to humans, exerts toxins that affect the kidneys, respiratory and skeletal systems (WHO, 2010).

2.1.2 Manganese

Mn is an essential heavy metal that is emitted into the environment through exhausts of automobiles and from dust that pollutes the air that is on the same level as the ground. As an essential heavy metal, it is found commonly in the environment and is integrated in the diet as it is required for the maintenance of good health when consumed in very low amounts. According to the EPA, when the concentration of Mn crosses the value of 300 ppm, it imposes a serious threat to the human health (Horneck, Sullivan, Owen, & Hart, 2011).

Exposure to low levels of Mn over a lifetime was found to increase the exposure of individuals to parkinsonism. It also has neurological effects that are less pronounced but could be anger, confusion, tension, as well as reduced coordination between the hands and eyes (Pavilonis et al., 2014).

2.2 BIOREMEDIATION

Bioremediation is defined as the action of employing organisms that occur naturally to remove or neutralize many contaminations from waste into non-toxic forms, while maintaining that no toxic or harmful chemicals are used in this type of remediation of soil. And there have been many current advances in the field of bioremediation over the past twenty years that point to one main goal, which is the ability to use eco-friendly approaches to restore contaminated environments effectively (Azubuike, Chikere, & Okpokwasili, 2016).

Indigenous microorganisms that occur naturally present in the contaminated environments are what have the power to fixing many of the issues that are connected with bioremediation of pollutants (Verma and Jaiswal 2016) while maintaining that optimal conditions in the environment are available for their appropriate growth and metabolism. Many techniques of bioremediation have been developed by researchers where microorganisms adopt a variety of mechanisms that detoxify the soil such as biosorption, biodegradation, bioaccumulation and biotransformation either *in situ* or *ex* situ (Satinder et al., 2006).

Bioremediation is a process that relies on many biological operations like degrading, mineralizing, detoxifying or transforming to decrease the pollutants' concentration to a non-toxic level (Azubuike, Chikere, & Okpokwasili, 2016). The procedure of removing pollutants depends mainly on the pollutant itself and its nature, and that includes: chlorinated compounds, agrochemicals, dyes, heavy metals, plastics, greenhouse gases, hydrocarbons, sewage, and nuclear waste. Removing pollutants also depends on the site of pollution, where in this case we must categorize the techniques of bioremediation into either in situ or ex situ (Frutos et al. 2012; Smith et al. 2015).

Heavy metals are mere atoms at the most fundamental level and are generally considered natural element whether essential or non-essential, which is why metabolism and degradation are impossible. Microorganisms have instead progressed strategies that help them cope with transforming an element to a less harmful or non-toxic form, restraining the metal extracellularly or intracellularly. Therefore, it prevents it from causing any interactions that are harmful to the host cell (White & Gadd, 2000).

2.3 MYCOREMEDIATION

Mycoremediation is another face of bioremediation that depends on the presence of native fungi (microorganism) in the site of pollution. It is a process where fungal mycelium is used to take on the role of degrading the pollutants in the site of pollution. And for that reason, mycoremediation is considered as the single most complex type of bioremediation (Thakur, 2014).

Mycoremediation works on converting a polluted and toxic site into a less toxic one in two ways, either in situ or ex situ. In in situ approach, the soil is treated with fungi on site without relocation and the factors that affect the process of mycoremediation are the environmental conditions like temperature and the pH as well as other factors like the nutrient availability and the moisture content (Folch, Vilaplana, Amado, Vicent, & Caminal, 2013). In ex situ approach, the soil is removed physically and transferred to another location for treatment and the determination of the technique depends on the depth, degree and kind of pollutant (Philp and Atlas 2005).

2.4 FUNGI

Fungi have an enzymatic machinery that allows them to be used for the purpose of degrading heavy metals and other types of waste using many processes like biosorption and bioconversion (Kulshreshtha, Mathur, & Bhatnagar, 2014). The fungus is mostly dependant on the strength and components of its cell wall because of its important role as the interactant part of the fungi with its environment. That interaction is what makes fungi a great biosorbent in the process of biosorption. The process of biosorption mostly depends on the large surface area and the polarity of the cell wall of the fungi so that more binding of metal occurs to the functional groups on the cell wall and so the fungi's ability of metal sequestration increases (Dhankhar & Hooda, 2011).

2.4.1 Fungi tolerance to Cd

A study by Mohammadian Fazli et al. (2015), identified seven fungi that are highly tolerable to heavy metals such as *Terichoderma* sp, *Paecilomyces* sp.G, and *Aspergillus fumigatus* but the most tolerable amongst them was reported to be *Aspergilus versicolor* that had the highest tolerance index of 0.85. It also showed active growth in the presence of Cd on agar media, and red pigments on the media proved the that the Cd could bind to the protein content of the mycelium's cell wall which happens if the fungi have a true bioremediating ability. In another research that was done by Mohsenzadeh, and Shahrokhi (2014), the removal of Cd at different concentrations by the fungal species of *Trichoderma* showed successful results of high absorption rates and high uptake of Cd. The removal and uptake capability of *T. asperellum* were 80.37% of removal at 100 ppm of Cd, while *T. tomentosum* had a removal of 76.98% at 100 ppm.

2.4.2 Fungi tolerance to Mn

A research that was done by Wu, Xu, Ding, Li, and Xu (2016) to study the mycoremediation of Mn by *Pleurotus eryngii* mycelium showed that there was a significant percentage of removal of Mn by *Pleurotus eryngii* with a value of 82.27% at 2 mM of Mn.

2.5 MEDIA

PDA (Potato Dextrose Agar) is a medium that is used for general and common purposes such as the identification and cultivation of molds and yeast either in foods or clinical samples as recommended by the FDA. It can also be used for the purpose of stimulating sporulation and the culturing and distinguishing of non pathogenic and pathogenic fungi. PDA contains potato infusion that supplies the media with the nutrient foundation for the optimal growth of fungi, and dextrose that is a source of carbohydrates and stimulate the growth of fungi, while agar is the agent that helps in solidifying the media (Rijal, 2015).

PDB (Potato Dextrose Broth) is another type of media without agar that is recommended by the FDA to be used for the stimulation of sporulation and identification of fungi as it is mainly used for the preparation of inoculum that will then be used to be smeared on the agar medium. PDB contains both dextrose and potato infusion that provide the required nutrients for the optimal growth of fungi ("Potato Dextrose Broth", 2017).