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# Bioremediation of Heavy Metals by using *Aspergillus niger* and *Candida albicans*

Muzhda Qasim Qader* , Yahya Ahmed Shekha

Department of Environmental Science and Health, College of Science,Salahaddin University- Erbil, Iraq

**A B S T R A C T:**
Bioremediation is a branch of biotechnology that employs the use of living organisms, like microalgae and fungi, in the removal of contaminants, pollutants, and toxins from soil, water, and other environments. The study was design to know and evaluate the efficiency of fungi to remediate two types of heavy metals (Pb and Cd), by using different concentrations (5, 15, 35, and 50ppm). Results revealed that the lowest applied dose (5ppm) of both tested heavy metals had the highest reduction percent by using two fungal stains *Aspergillus niger* and *Candida albicans* were remove lead by 85.6 and 84.2%, while for cadmium were 80 and 78.4% respectively. Statistically significant differences ($P \leq 0.05$) were observed between control and the treatments for both tested heavy metals. Highest heavy metals removal was found at the end of experiment (20 days).

KEY WORDS: Mycoremediation, Bioremediation, *Aspergillus niger*, *Candida albicans*, Heavy metals.
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## 1.INTRODUCTION:

Biotechnology is a methodology that makes use of living organisms to create new products, improve their quality, and reduce human suffering (Olatunji et al., 2007). Biotechnology is described as the use of biological structures, living creatures, or their derivatives in the development and transformation of processes for better and more specific applications, as well as the end products that can be used for particular purposes (Fletcher, 1992).

Microorganisms destroy pollutants through their metabolic activities, and this ability of microorganisms has been used in the development of biotechnologies. Microorganisms are also capable of degrading hazardous natural wastes such as polycyclic aromatic hydrocarbons pesticides, polychlorinated biphenyls metals, nitrogen compounds, halogenated organic solvents and chemicals, non-chlorinated insecticides and herbicides, and radio nuclides (Leung et al., 2019).

* **Corresponding Author:**
**Muzhda Qasim Qader**
E-mail: **Mzhda.qadir@su.edu.krd**
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Heavy metals release into the environment has been steadily rising, as a result of human activity and technological development, due to accumulation in the food chain, their toxicity and persistence in nature, pose a serious threat to the environment and public health. The main causes of the rise of metallic species released into the environment include mining operations, agricultural runoff, industrial, and domestic effluents (Sala Cossich et al., 2002).
Heavy metal (s) are common pollutants that cause a serious environmental problem since they are persistent and non-degradable. Each metal has a permissible limit, outside which they are generally toxic and some of them even hazardous (Gupta et al., 2000). Heavy metals are a serious environmental concern, due to the fact that they are toxic, have a tendency to bioaccumulate, and pose a risk to both human life and the environment (Eroğlu and Şeker, 2007, Igwe and Abia, 2006).

Mycoremediation has the potential to be, an economic, and eco-friendly, successful solution to an ever problems of soil and water pollution. Due to their robust growth, extensive hyphal network, production of adaptable extracellular ligninolytic enzymes, high surface area to volume ratio, resistance to heavy metals, ability to adapt to changing pH and temperature, and presence of metal-binding proteins, fungi are an excellent candidate for the remediation of various pollutants. (Khan et al., 2019, Singh et al., 2015, Kapahi and Sachdeva, 2017, Bhattacharya and Das, 2011).It can be used to remediate numerous contaminants produced by different industries, like dyes, herbicides, and pharmaceutical drugs, in-situ. As an alternative, it can be used in bioreactors. Bioreactors are systems that have their physicochemical properties constantly maintained to promote microbial growth (Aragão et al., 2020).

Various harmful compounds can be degraded by fungi. Changing local soil and water conditions can aid in fostering biological activity and, as a result, the degradation/removal of toxic substances via mycoremediation (Singh and Gauba, 2014). Fungi have also been proven to be effective in the removal of heavy metals such as lead (Pb) and cadmium (Cd). Fungi may accumulate these metals from water and store them in their tissues because they are already in their simplest condition and will not be degraded further (mycelia or fruiting mushroom bodies). After being utilized in mycoremediation, mushrooms used for this purpose must be treated as hazardous waste (Singh and Gauba, 2014).

Because heavy metals cannot be decomposed or destroyed, they are stable and persistent environmental contaminants. As a result, their toxicity poses significant environmental and health risks, requiring a continual search for efficient, cost-effective technology for detoxification of metal-contaminated sites, so by using microorganisms such as fungi this can be solved. The aim of the study is to evaluate the efficiency of two types of fungi in metal detoxification

## 1. MATERIALS AND METHODS

### 2.1 Experimental set-up and fungal cultivation

The fresh culture of selected fungi in this study has been sub-cultured individually onto slants containing solidified SDA Sabouraud's Dextrose Agar and incubated for 7 days at 25± 1 °C for *A. niger*. Spores of *A. niger* have been suspended from 7days old fresh culture 10 ml of sterile distilled water. To obtain similar cell counts of $3\times10^4$ CFU/ ml, spore suspensions have been diluted with double-sterile water. The fresh culture for *C. albicans* has been sub cultured individually onto slants containing solidified Sabouraud Dextrose Agar (SDA) and incubated at 37°± 1°C for 24 hrs., yeast cells had been suspended in 10 ml sterile distilled water. Double-sterile water has been used to dilute yeast cells to get similar cell counts of $2\times10^4$ CFU/ ml.

*Aspergillus niger* (ATCC 16404), and *Candida albicans* (ATCC 10231)(Ismael et al., 2019), were used as test organisms for remediation of heavy metals. Experiments were performed as batch reactors in Erlenmeyer glass flasks on a horizontal orbital shaker at 150 rpm at 27 °C. remediation experiments. To obtain sufficient biomass for the subsequent growth, *Aspergillus niger* (*A. niger*) was cultured in the SDB and grew for 7 days while *Candida albicans* (*C. alicans*) was cultures on SDB for 24 hrs before applying for bioremediation of heavy metals.

### 2.2 Heavy metal analysis

$PbCl_2$ and $CdCl_2$ were used to prepare the 1000 ppm stock solutions of Pb (II) and Cd (II) in deionized water. The heavy metal stock solution was added to the sterilized Saborauds Dextrose Broth (SDB) medium in 500 ml conical flasks. On SDB medium, 50 ml each fungal isolates were inoculated which has different concentrations of 5, 15, 35, and 50 ppm of the two heavy metals. These flasks were incubated on the shaker at 150 rpm at 28°C and inoculated.

Heavy metals were measured by lead and cadmium before and after treatment with fungus by using an atomic absorption spectrophotometer (AAS Perkins Elmer USA 1100D) (APHA, 2012), was used for laboratory measurement. The percentage of removal of metal concentrations was calculated according to (Al Ahmed, 2014 ).

$$Removal\ \% = [\frac{Initial\ conc. - Final\ conc.}{Initial\ conc.}] * 100$$

## 2.3 Statistical analysis

Statistical analysis was conducted for the data using a software program (SPSS version 26) and Excel spreadsheets. Two-way ANOVA (Analysis of variance). A post hoc test (multi comparisons Duncan test) was applied to determine significant differences at 0.05. All data are expressed as Mean ± SE (Marcello pagano 2018).

## Result and discussion

The mean value of doses and doses with times of heavy metals (5, 15, 35, and 50 ppm) by using *Aspergillus niger* and *Candida* sp. during 20 days of treatment shown in (Table 1 & Fig. 1-4).

The concentration of Pb by *Aspergillus niger* on day 20$^{th}$ of the experiment decreased and reached (0.71, 2.2, 6.2 and 10.8ppm), respectively, while for Cd were (1, 3.11, 7.8 and 11.6 ppm), respectively (Figure 1). It may be due to the ability of fungi to secrete large amounts of extracellular enzymes could have a significant impact on their ability to grow on a different hydrocarbon source (Steffen et al., 2003), in addition to their ability to bio convert heavy metals, they can also tolerate high levels (Lubertozzi and Keasling, 2009), bio absorbs (Abdullahi et al., 2017), bioaccumulate (Lubertozzi and Keasling, 2009) the metal ions. Baldrian, (2003), reported that the fungi have efficient mechanisms to reduce heavy metals (Cd, Fe, Hg, and Zn) by adsorption on the walls or formation of heavy complexes sedated and storing elements within their cells (Baldrian, 2003).

In addition Hajdu-Rahkama (2014), illustrate to binding of a greater number of heavy metals, and the use of functional groups on their cell walls which are characterized by the largest surface area. Fungi growing in situations with high levels of heavy metals have been reported to use a variety of such mechanisms. Metal sequestration or accumulation is one of them (Bello and Abdullahi, 2016) and enzymatic modification of the metal ions to a less toxic form (Abdullahi et al., 2017).

Barros Júnior et al., (2003), observed that the Cadmium biosorption with *Aspergillus niger* could be used successfully in the oil industry to remove heavy metals from oil field water (Barros Júnior et al., 2003). Bio-removal of Pb concentrations by *Candida albicans* on day 20$^{th}$ of the experiment were (0.79, 2.5, 6.7 and 11 ppm), respectively, whereas for Cd were (1.08, 3.3, 8.1 and 14), respectively (Figure 3&4). *Candida* sp. is a yeast candidate as indicator fungi for water pollution (Qadir, 2019), has high resistance to environmental conditions, and is highly biodegradable to a variety of resistive and organic poisons. According to a study, *Candida spp.* isolated from sewage samples can accumulate substantial amounts of Ni (57–71%) and Cu (52–68%) (Dönmez and Aksu, 2001). It was observed that *C. tropicalis* was able to remove 40% of the cadmium from the wastewater after 6 days and 78 % after 12 days (Rehman et al., 2011). Moctezuma Zárate et al. (2017), commented that *Candida albicans* could be used to decontaminate lead-polluted aquatic habitats.

In different habitats, different populations of yeast can be found, and changes in the community reflect changes in the environment (Qadir, 2019). *Candida* sp., *Rhodotorula* sp., *Trichosporon* sp., *and Cryptococcus* sp. are suggested to be indicator fungi for water contamination (Dynowska, 1997). Furthermore, yeasts are highly biodegradable to a variety of resistive and organic poisons (Qadir, 2019).

**Table 1**: Bioremediation of heavy metals by using *A. niger* and *C. albicans*. Data represented as (Mean ± SE).

| Metal concentrations | *Aspergillus niger* | | *Candida albicans* | |
|---|---|---|---|---|
| | **Lead** | **Cadmium** | **Lead** | **Cadmium** |
| 5ppm | 3.08±0.144$^{d}$ | 3.37±0.119$^{d}$ | 3.13±0.131$^{d}$ | 3.41±0.117$^{d}$ |
| 15ppm | 10.12±0.144$^{c}$ | 11.16±0.119$^{c}$ | 10.36±0.131$^{c}$ | 11.55±0.117$^{c}$ |
| 35ppm | 22.84±0.150$^{b}$ | 23.65±0.124$^{b}$ | 23.51±0.137$^{b}$ | 23.92±0.185$^{b}$ |
| 50ppm | 29.79±0.141$^{a}$ | 33.90±0.117$^{a}$ | 30.18±0.129$^{a}$ | 34.49±0.174$^{a}$ |

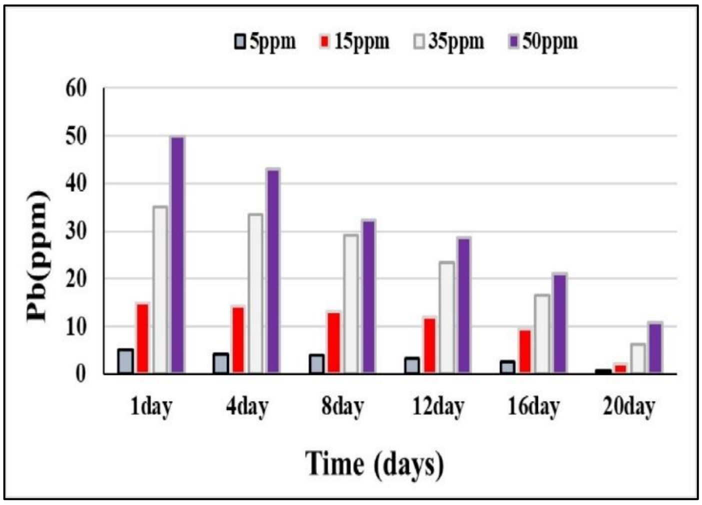


**Figure 1**: Bioremediation of different Lead concentrations by *Aspergillus niger*.

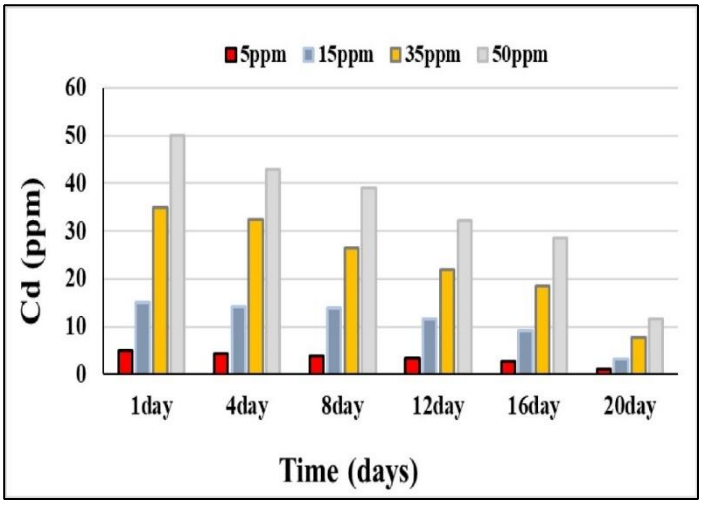


**Figure 2**: Bioremediation of different Cadmium concentrations by *Aspergillus niger*.

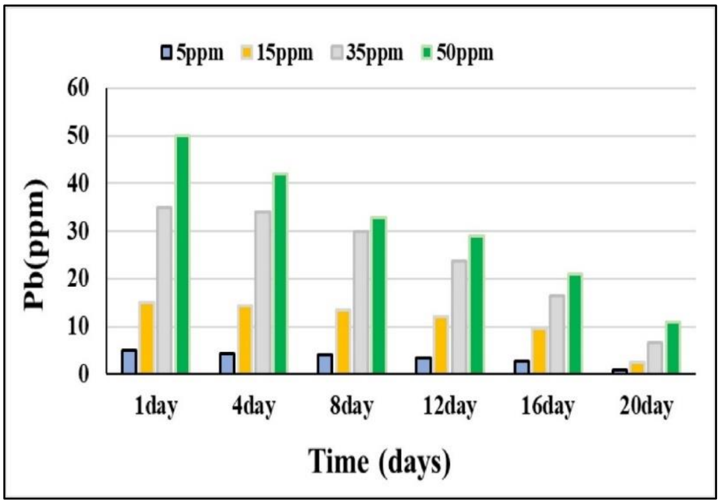


**Figure 3**: Bioremediation of different Lead concentrations by *Candida albicans*

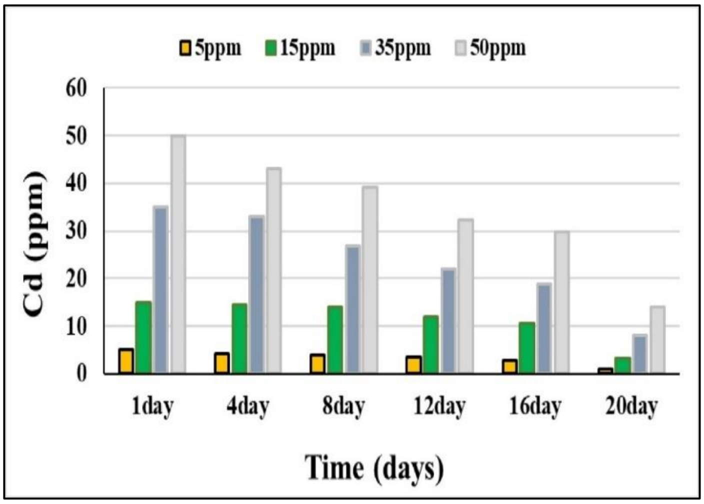


**Figure 4**: Bioremediation of different Cadmium concentrations by *Candida albicans.*

The results of Acosta-Rodríguez et al. (2018b), revealed that Cobalt (71.4%) and fluor (83%), mercury (83.2%), and zinc (100%) were the heavy metals that fungus removed most effectively, but silver (I) (48%) and copper were less effective (37 % ).

The ability of the biomass of *A. niger* to remove heavy metals including mercury, cadmium, and copper is equal to or greater than that of other biomasses that have been investigated. It was observed that after 7 days of incubation, using planktonic *R. mucilaginosa* cells for 48 hours, 71 %, 69 %, and 96.4 % of the heavy metals present in the contaminated water were eliminated (4.79 %, 10.25 %, and 5.49 %, respectively) (Acosta-Rodriguez et al., 2017). Lead, zinc, cadmium, and copper were removed in two steps by *A. niger* at rates of 84.3 %, 84.4 %, 25 %, and 14.4 %, respectively (Yang et al., 2009b). In batch systems, immobilized *A. niger* cells removed 95 % of the cadmium (II), 88 % of the lead (II), 70 % of the iron (III), 60 % of the copper (II), 48.9 % of the nickel (II), 37.7 % of the manganese (II), and 15.4 % of the zinc (II) from industrial wastewater (Tsekova et al., 2010). After six days, *C. tropicalis* was able to remove 40% of the cadmium from the wastewater and after 12 days, it was able to remove 78 % (Rehman et al., 2011).

The present study showed that *Aspergillus niger* has a higher removal percent for all concentrations of Pb were removed by (85.5, 85.33, 82.28, and 78.4%) and for Cd were (80, 79.26, 77.71, and 76.8%) respectively, whereas percent reduction of Pb by *Candida albicans* for Pb, were (84.2, 83.3, 80.85 and 78%) while for Cd (78.4, 78.76.85 and 72 %) respectively on day $20^{th}$ (Figure 5 & 6). According to the present study, the lowest applied concentration (5ppm) of heavy metals (Pb and Cd) has the highest reduction percent for both fungal species *A. niger* and *C. albicans*.

Fungi can tolerate heavy metal compounds because they absorb metal ions and chelate them on the cell surface (Gadd and White, 1993, Anahid et al., 2011). Heavy metal detoxifications are frequently accomplished through compartmentalization the metal and bio transforming it into an inactive form (Nies, 1999, Nies et al., 1989). It has also been demonstrated that fungi have unique genes for resistance to heavy metal ions. Most of these genes are chromosomal, but some are plasmids borne (Nies, 1999). The presence of specific metal ions mostly induces the plasmid encoded genes (Rosen, 2002). Based on these, bioaccumulation is a mechanism through which fungi resist heavy metal ions (Machido et al., 2014).

Moreover, fungi have a variety of mechanisms that make them efficient in the process of reducing heavy metal concentrations, such as adsorption on the walls of external fungal adsorption or the formation of heavy complexes sedated and storing elements within their cells, fungi play an important role in reducing and removing heavy metal concentrations in the soil and aquatic environment (Siddiquee et al., 2015, Dhaliwal et al., 2020).

Researchers have researched the ability of fungi to remove heavy metals. *A. niger* removed copper, cadmium, and lead with percentages of 84.3, 84.4, and 25 %, respectively (Yang et al., 2009a). After 12 days, C. tropicalis was able to remove 78% of the cadmium from the wastewater (Rehman and Anjum, 2011, Acosta-Rodríguez et al., 2018a). The different metal sensitivities of the isolated fungal species are suggested by the differences in resistance to Pb, Cr, and Cd observed among the fungal genera isolated from the study sites (Nies and Silver, 1995, Shazia et al., 2012).

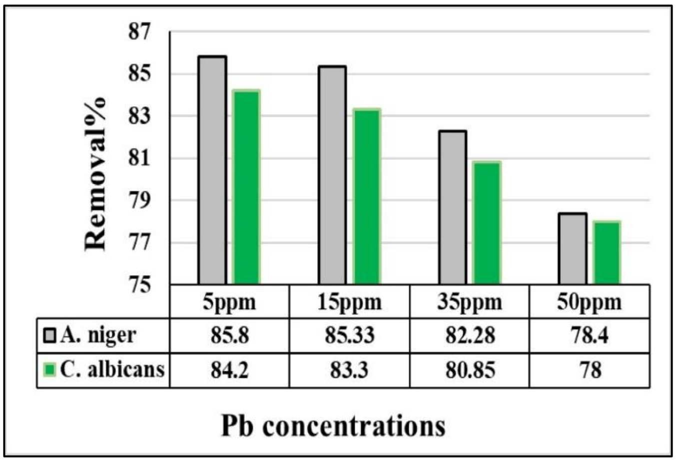


**Figure 5**: Removal percent of different Lead concentrations by two fungal strains.

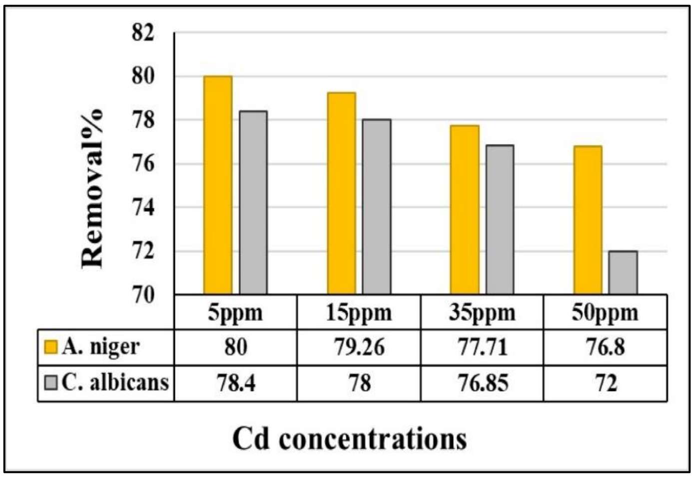


**Figure 6:** Removal percent of different lead concentrations by two fungal strains.

## 2. CONCLUSIONS

Fungi play an essential role in different environments as decomposers of different substances, they possess high tolerance potential for heavy metals due to their increasing ability to adapt to contaminants over time. It was found from the current study that both types of fungi were more efficient to remove lead compared to cadmium. Also, *Aspergillus niger* was more effective for remediation both components for all tested concentrations compared to the *Candida albicans*, and the highest removal rate was at concentration of (5 ppm) and the removal rate decreased with the increase in the concentration of the two heavy metals.

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