

REDUCING CONCENTRATIONS OF CERTAIN HEAVY METALS IN WASTEWATER USING NON-LIVING FUNGAL BIOMASS

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ABSTRACT

Four fungi dead biomass *Aspergillus terries*, *Aspergillus niger*, *Rhizopus oligosporium*, *Rhizopus raezea* were used to reduce heavy metals concentrations of (Pb 0.67 ppm, Cd 0.122 ppm and Cu 0.96 ppm) from final discharge of Khartoum Wastewater Treatment Plant in laboratorial conditions of (25 °C, period treatment of 24 hr). Dead biomass showed high ability to reduce Pb concentration about 16.4-100% and Cd about 19.6-100% and Cu concentration about 18.7-100% in 24 hr period treatment and highly significant differences $P < 0.01$. Dead biomass of *Aspergillus niger* showed more ability to reduce Pb, Cd and Cu concentrations in 100%, While, the dead biomass of *Rhizopus raezea* showed less ability to reduce heavy metals elements in 16.4-19.67%.

KEYWORDS: Wastewater, treatment, fungi, heavy metals.

INTRODUCTION

Industrial waste, agricultural runoff, and sewage are major sources of aquatic environmental pollution—introducing various organic and inorganic pollutants as well as heavy metals—when discharged into surface waters without adequate treatment. These pollutants alter natural water characteristics, with heavy metals posing a particular threat due to their hazardous toxic effects on the environment (Korboule et al., 2007). Undoubtedly, the entry of heavy metals into the aquatic environment disrupts the ecosystem's balance and poisons aquatic plants, fish, and other organisms; furthermore, these elements are highly toxic to humans once they enter the food chain (Borgmann, 2005). Filamentous fungi play a significant role in reducing and removing heavy metal concentrations from soil and aquatic environments. They achieve this through various efficient mechanisms, such as adsorption onto the outer fungal mycelial walls, the formation of insoluble heavy metal complexes, biosorption, and intracellular accumulation (Guibal et

al., 2001; Balderian, 2009). Studies by Bennet et al. (2008) have demonstrated the substantial role and high efficacy of fungi in the bioaccumulation of heavy metals, highlighting the mycelium's ability to efficiently adsorb cadmium, iron, mercury, and zinc in aquatic environments. The efficiency of fungal mycelium in metal adsorption stems from the composition of the hyphal cell wall, which consists of compounds such as polysaccharides and proteins. The latter contain functional groups—including carboxyl, hydroxyl, phosphate, and amino acid groups—that facilitate binding with heavy metal ions (Gadd, 1992; Veglio and Beolchini, 2008). This study aims to evaluate the efficiency of non-living mycelial biomass from the fungi **Aspergillus terreus**, **Aspergillus niger**, **Rhizopus oligosporus**, and **Rhizopus oryzae** in reducing concentrations of heavy metals—specifically lead, cadmium, and copper—from sewage wastewater under laboratory conditions.

MATERIALS AND METHODS

1- Sample Collection Method

Samples of final effluent were collected from the Khartoum Wastewater Treatment Plant using clean, 2-liter polyethylene bottles; the bottles were tightly sealed and transported to the laboratory. The samples were vacuum-filtered using 0.45-micron pore-size filter paper and sterilized in an autoclave at 126°C and 1.5 bar pressure. Additionally, two samples of the final effluent were collected in sterile, opaque 100-milliliter glass bottles for the purpose of fungal isolation and identification.

2- Isolation, Purification, and Identification of Fungi

Four fungal species—*Aspergillus terreus**, *Aspergillus niger**, *Rhizopus oligosporus**, and *Rhizopus oryzae**—were isolated, cultured, and identified from the final effluent of the wastewater treatment plant, following the method of Romero et al. (2005). Malt Extract Agar (MEA) medium was prepared and supplemented with the antibiotic chloramphenicol (prepared by dissolving 250 mg of the antibiotic in 250 mL of distilled water). One milliliter of the wastewater sample was placed into sterile glass Petri dishes (9 cm diameter), and the sterilized solid culture medium was added; the dishes were swirled gently to mix the contents. The plates were incubated at 25°C for 48 hours, and the appearance of fungal colonies was monitored. Microscope slides were prepared to identify the fungal species using the taxonomic key provided by Webster (1980).

3- Preparation of Fungal Biomass (Non-living)

Pure fungal cultures grown in liquid mineral broth were killed using an autoclave at 121°C and 1.5 bar pressure

for 20 minutes. Three-day-old colonies were selected for *Aspergillus terreus**, *Aspergillus niger**, and *Rhizopus oligosporus**, while five-day-old colonies were selected for *Rhizopus oryzae**. The dead fungal biomass was washed with sterile distilled water, dried on filter paper within sterile glass Petri dishes, and weighed using a sensitive balance (Roane et al., 2009).

4- Analysis of heavy metals (lead, cadmium, and copper)

Sterile 100 mL glass flasks were used, each containing 50 mL of sterilized final effluent from the wastewater treatment plant and the prepared fungal biomass (at a rate of 795 mg per fungal isolate, in triplicate). The flasks were incubated at 25°C for 24 hours at a pH ranging from 6.5 to 7.6. Concentrations of lead, cadmium, and copper were measured before and after fungal treatment using an atomic absorption spectrophotometer, following the APHA (1998) method; sample pH was measured using a pH meter. The percentage reduction in element concentrations was calculated as follows:

$$\% = (\text{Initial Concentration} - \text{Final Concentration}) / \text{Initial Concentration} \times 100\%$$

5- Statistical analysis

Results were statistically analyzed using Analysis of Variance (ANOVA) and the SPSS statistical software package (Al-Aqili, 1998).

RESULTS AND DISCUSSION

Table (1) illustrates the reduction in concentrations of certain heavy metals—lead, cadmium, and copper—from the final discharge basins of Khartoum Wastewater Treatment the plant, both before and after treatment with non-living fungal biomass.

Table (1): Reduction in concentrations of heavy metals (lead, cadmium, and copper) using non-living biomass of the fungi *Aspergillus terreus, *Aspergillus niger**, *Rhizopus oligosporus**, and *Rhizopus oryzae**.**

	Element	Concentration before treatment (ppm)	Concentration after 24 hours of treatment (ppm)			
			<i>As. niger</i>	<i>As. terries</i>	<i>R. oligosporium</i>	<i>R. raezea</i>
1	Lead	0.67	0	±0.04*0.02	±0.059**0.001	±0.56**0.001
2	Cadmium	0.122	0	±0.03*0.02	*0.003 ±0.029	±0.098*0.002
3	Coppe	0.96	0	*0.02±0.08	**0.002±0.076	**0.002±0.78

Results represent the mean ± standard deviation.

** Represents highly significant differences (P<0.01) compared to pre-treatment.

* Represents significant differences (P<0.05) compared to pre-treatment.

The results demonstrated that the non-living biomass of the fungus *Aspergillus niger** was highly efficient in removing certain heavy metal concentrations from wastewater within a 24-hour treatment period, with high significance (P<0.01). The non-living biomass of *Aspergillus terries** showed efficiency in reducing the concentration of lead to (0.04 ± 0.02), cadmium to (0.03 ± 0.02), and copper to (0.08 ± 0.02), with high significance (P<0.01). The non-living biomass of *Rhizopus oligosporium** was efficient in reducing heavy metal concentrations to (0.059 ± 0.001) for lead (significance P<0.05) and to (0.029 ± 0.003) for

cadmium (high significance P<0.01), and in reducing the copper concentration to (0.076 ± 0.002). Significant reductions (P<0.05) were observed after 24 hours of treatment. Meanwhile, the non-living biomass of the fungus *Rhizopus oryzae** demonstrated lower efficiency in reducing elemental concentrations; lead levels dropped to 0.56 ± 0.001 (P<0.05), cadmium levels to 0.098 ± 0.002 (highly significant, P<0.01), and copper levels to 0.78 ± 0.002 (P<0.05) within the 24-hour treatment period. These results align with the findings of Omotayo et al. (2008) regarding the efficiency of non-living biomass from fungi such as *Aspergillus niger**,

*Penicillium austurianum**, *Saccharomyces cerevisiae**, and *Mucor circinelloides** in reducing heavy metal concentrations in contaminated soils; their study demonstrated that these isolates reduced iron concentrations by 60–75% and showed that direct contact between the selected fungal hyphae and the wastewater sample effectively facilitated the adsorption of heavy metals onto the hyphal cell walls. The results also align with the findings of Iqbal et al. (2009) regarding the removal of chromium and cadmium from industrial wastewater using non-living biomass of *Aspergillus** and *Rhizopus** fungi; they demonstrated the ability of these fungi to remove these heavy metals from liquid cultures within 18 hours. Similarly, the results are consistent with the study by Teskova and Petrov (2008) on *Rhizopus delemar**, which showed the high efficiency of its biomass in reducing metal concentrations in liquid culture.

A 100% reduction in lead, cadmium, and copper concentrations was achieved using the non-living biomass of *Aspergillus niger** over a 24-hour treatment period. Regarding lead, the reduction rates were 94.03%, 91.19%, and 16.4% for the non-living biomass of *Aspergillus terreus**, *Rhizopus oligosporus**, and *Rhizopus oryzae**, respectively. For cadmium, the reduction rates were 75.41%, 76.22%, and 19.67% for the respective fungal biomasses. Finally, for copper, the reduction rates were 91.67%, 92.08%, and 18.755% for *Aspergillus terreus**, *Rhizopus oligosporus**, and *Rhizopus oryzae**, respectively. The results demonstrate the high capacity of *Aspergillus niger** biomass to reduce concentrations of lead, cadmium, and copper by 100% within a 24-hour treatment period. This is attributed to the fungus's cohesive mycelium and substantial biomass (973.4 mg); direct contact with the wastewater sample increased the surface area available for the adsorption of heavy metals onto the outer walls of the mycelium—a phenomenon also confirmed by Frey and Brunner (2009) in their study on mycorrhizal fungi and their ability to adsorb aluminum from contaminated soil onto mycelial cell walls. These findings align with the study by Bai and Abraham (2008) on *Rhizopus nigricans**, which demonstrated the fungus's ability to remove chromium from liquid culture; the mycelium spread extensively throughout the medium, thereby increasing the surface area for adsorption. Similarly, Daniel (2006) observed the capacity of *Armillaria** biomass to significantly reduce concentrations of zinc, lead, and cobalt in contaminated soil over extended periods through bioaccumulation, while a study by Preetha and Viruthagiri (2009) demonstrated the high efficiency of *Rhizopus** biomass in reducing zinc concentrations in liquid culture. The results indicated that the non-living biomass of the fungus *Rhizopus raezea** was less efficient in reducing concentrations of lead, cadmium, and copper. This was due to the lack of cohesion in its mycelium and the formation of a low-density, thin mass, which resulted in poor contact with the water samples; the thickness and cohesion of the

mycelium play a significant and effective role in the adsorption of heavy metals. This finding is supported by Abd Askar, et al. (2025) in their comparative study on the efficiency of non-living biomass from the fungi *Rhizopus arrhizus**, *Mucor miehei**, and *Penicillium chrysogenum** in reducing heavy metal concentrations in contaminated soils.

CONCLUSIONS

- 1- The non-living biomass of the fungi *Aspergillus terreus**, *Aspergillus niger**, and *Rhizopus oligosporus** demonstrated the ability to reduce concentrations of the heavy metals lead, cadmium, and copper in wastewater samples, achieving high reduction rates of 75–100%.
- 2- The non-living biomass of the fungus *Aspergillus niger** exhibited high efficiency, reducing heavy metal concentrations by 100% within a 24-hour treatment period.
- 3- The non-cohesive, low-density biomass of the fungus *Rhizopus raezea** showed lower efficiency in reducing concentrations of lead, cadmium, and copper, achieving reduction rates of 16–19% within a 24-hour treatment period.

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