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*By Universitas Muhammadiyah Sidoarjo*

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## **Fungi as a Sustainable Solution for Heavy Metal Removal in Wastewater Treatment**

*Jamur sebagai Solusi Berkelanjutan untuk Menghilangkan Logam Berat dalam Pengolahan Air Limbah*

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### **Abstract**

This study investigates the ability of non-living fungal biomass from *Aspergillus terreus*, *Rhizopus oligosporus*, and *Rhizopus arrhizus* to reduce heavy metal concentrations in wastewater. Using non-living fungal blocks, the experiment targeted the removal of lead (0.70 ppm), cadmium (0.110 ppm), and copper (0.80 ppm) from wastewater at the Al-Dur district sewage treatment station. Conducted under controlled conditions (25 degrees Celsius, pH 6.5-7.6) over a 24-hour period, the results demonstrated significant reductions in metal concentrations, with statistical significance at  $P < 0.01$ . These findings suggest that non-living fungal biomass could be a cost-effective and environmentally friendly alternative for heavy metal remediation in wastewater treatment.

#### **Highlights:**

- Fungal biomass effectively reduces lead, cadmium, copper quickly.
- Results statistically significant, demonstrating reliability.
- Sustainable, cost-effective alternative to chemical treatments.

**Keywords:** Heavy Elements, *Aspergillus Terries*, *Rhizopus Oligosporium*, Fungal Biosorption, Non-Living Biomass

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## Introduction

Industrial waste, agricultural waste, and wastewater are among the main sources of pollution of the aquatic environment with many organic and inorganic pollutants and heavy metals when thrown into surface water without proper treatment, as these pollutants change the characteristics of natural water, and heavy metals are considered dangerous pollutants due to their dangerous toxic effects. on the environment [1]

Filamentous fungi play an effective role in reducing and removing the concentrations of many heavy elements in the soil and aquatic environment because they possess a group of different mechanisms that make them efficient in the process of reducing the concentrations of heavy elements, including the adsorption process on the external mycelium walls, the formation of precipitated heavy complexes, or the mechanism of biological drinking. Biosorption and storage of elements within their cells [2][3] showed in their study that fungi have a major role and high effectiveness in the process of bioaccumulation of heavy metals and the ability of mycelium to absorb cadmium, iron, mercury, and zinc with high efficiency in the aquatic environment.

The efficiency of mycelium in absorbing metals results from the structure of the cell wall of the fungal hyphae, which consists of a group of compounds such as polysaccharides and protein. The latter is composed of secondary groups such as carboxyl and hydroxyl, as well as contains phosphate and groups of amino acids. All of these groups of materials work to bind with... Heavy element molecules [4][5].

The current study aims to study the efficiency of mycelium mycelium of non-living masses of the fungi *Aspergillus terries*, *Rhizopus oligosporium*, and *Rhizopus raezea* in reducing concentrations of heavy metals such as lead, cadmium, and copper from sewage wastewater in the laboratory.

## Methods

### A. Sample Collection

Samples were collected from the Al-Dur city water station using opaque, sterilized glass bottles with a capacity of 3 liters, which were tightly closed and transported to the laboratory. The samples were filtered with a vacuum device using filter papers with a hole size of 0.45 microns and sterilized with an autoclave at a temperature of 126 and a pressure of 1.5 bar. Three samples of water from the Al-Dour water station were collected using opaque, sterile glass bottles with a capacity of 200 milliliters for the purpose of isolating and diagnosing fungi.

### B. Isolation, Purification and Diagnosis of Fungi

Three fungal species were isolated, developed, and diagnosed from the Al-Dur water station, namely *Aspergillus terries*, *Rhizopus oligosporium*, and *Rhizopus raezea*, according to the method [6], where the solid culture medium was prepared, Malt extract agar (MEA), to which the antibiotic chloromphenicol was added. By dissolving 250 mg of the antigen in 250 milliliters of distilled water, placing 1 milliliter of the Al-Dour city water sample in sterile glass dishes with a diameter of 9 centimeters, adding the sterile solid culture medium to it, shaking it well, incubating the dishes at a temperature of 25 degrees Celsius for 72 hours, and observing the appearance of fungal colonies. Glass slides were made for the purpose of diagnosing fungal species based on the taxonomic key[7].

### C. Examination of the Heavy Elements Lead, Cadmium and Copper

Sterile 100 ml glass beakers were used, in which 50 ml of Al-Dour city water station and the diagnosed fungal samples were placed for each fungal isolate and for three replicates. The beakers were incubated at 25 degrees Celsius for 24 hours with a pH value ranging between 7.6 - 6.5. The heavy metals lead and cadmium were measured. And copper before and after treatment with fungi using an atomic absorption spectrophotometer. The APHA (2020) method was used for laboratory measurement, and the pH value of the samples was measured using a pH meter. The percentage of reducing element concentrations was calculated as follows:

$\% = \frac{\text{initial concentration} - \text{final concentration}}{\text{initial concentration}} \times 100\%$

### D. Statistical Analysis

The results were analyzed statistically using the Analysis of Variance (ANOVA) test and using the statistical program SPSS[8].



## Results and Discussion

Table (1) shows the reduction in concentrations of some heavy metals - lead, cadmium, and copper - from the Al-Dour water station before and after treatment with fungal samples.

Table (1) Reducing the concentrations of heavy metals lead, cadmium, and copper using fungal samples *Aspergillus terries*, *Rhizopus oligosporium*, *Rhizopus raezea*

Chemical Element	Concentration Before Treatment (ppm)	Concentration After 24 Hours Treatment (ppm)		
		<i>R. raezea</i>	<i>R. oligosporium</i>	<i>As. terries</i>
Lead	0.70	*0.02 ± 0.03	**0.001 ± 0.057	**0.001 ± 0.59
Cadmium	0.110	*0.02 ± 0.04	*0.003 ± 0.024	*0.002 ± 0.096
Copper	0.80	*0.02 ± 0.07	**0.002 ± 0.074	**0.002 ± 0.77

**Table 1.**

Results represent mean ± standard deviation

\*\* Represents highly significant differences (P<0.01) compared to before treatment

\* Represents significant differences (P<0.05) compared to before treatment

The results showed that the non-living mass of the fungus *Rhizopus oligosporium* is efficient in reducing heavy metal concentrations to (0.057±0.001) for lead with a significant P<0.05 and for cadmium (0.024±0.003) with a high significance P<0.01 and reducing the copper concentration to (0.074±0.002). Significantly P<0.05 within 24 hours of treatment, while the non-living mass of the fungus *Rhizopus raezea* showed less efficiency in reducing element concentrations, as the concentration of lead decreased to (0.59±0.001) and significantly P<0.05, and cadmium decreased to (0.096±0.002) with high significance. P<0.01, and the copper element decreased to (0.77±0.002) and significantly P<0.05, within 24 hours of treatment. The results were consistent with [9] in their study on the efficiency of non-living masses of fungi. *Aspergillus niger*, *Penicillium austrianum*, *Saccharomyces cerevisiae*, *Mucor arcinoidoides* In reducing the concentrations of heavy metals from contaminated soil, they showed in their study the efficiency of these isolates in reducing iron concentrations by 60-75%, and that direct contact of the fungal hyphae selected for the study with the waste water sample had an effective effect in the process of adsorption of heavy elements onto the walls of the fungal hyphae.

The results also agreed with [10] in their study of removing chromium and cadmium elements from industrial waste water using non-living masses of *Aspergillus* and *Rhizopus* fungi, as they demonstrated the ability of these fungi to remove these heavy elements within 18 hours from liquid culture, as well as [11] in their study of the fungus *Rhizopus delemar* and the ability of its mass to reduce the concentrations of elements from the liquid culture with high efficiency. Figure (1) shows the percentage of reduction in the concentrations of heavy elements lead, cadmium and copper from the final discharge water sample of the wastewater treatment plant and the relationship with the non-living mass of the fungi. The elected

The percentage of reducing the elements lead, cadmium, and copper using the non-living mass of the fungi selected for the study within 24 hours of lead was 94.03%, 91.19%, and 16.4% for the non-living masses of the fungi *Aspergillus terries*, *Rhizopus oligosporium*, and *Rhizopus raezea*, respectively, and the percentage of reducing the concentration of the cadmium element was 75.41%. 76.22% and 19.67% for non-living fungal masses, respectively, and for the copper concentration, the reduction percentage was 91.67%, 92.08%, and 18.755% for *Aspergillus terries*, *Rhizopus oligosporium*, and *Rhizopus raezea*, respectively. At [12]demonstrated through a fact that the mass of *Rhizopus fungus* has a strong efficiency to lower the concentration of zinc in extractions. The result indicates that a mass which is not alive comes from *Rhizopus raezea*, it weighs 543 which represents 63. 86% of the total mass. While 2 mg of mercury is not as effective in reducing the levels of lead, cadmium, and copper like other more potent solvents, it does work to some degree. It is because of the production of a thin base with a low density and grasping quality to the water material which plays an insufficient role to separate the cells or molecules. The process of trapping the heavy metals is highly dependent upon the thickness and cohesion of the mycelium, which turns them bridging factors in this context. The majestic thing of this experiment was that the research that was done by [13]can prove beyond reasonable doubt that the dead masses of fungi *Rhizopus arrhizus*, *Mucor miechei*, and *Penicillium chrysogenum* are also useful in reducing levels of heavy metals in the contaminated soil.

## Conclusions

1.The ability of *Aspergillus terries*, *Rhizopus oligosporium*, a fair-sized fungus giant to reduce the lead, cadmium, and copper levels in the sample wastewater to environmentally acceptable levels with discharge permits at 75 -100 % concentrations was confirmed.



2. The not united and thin mass of the fungus *Rhizopus raezea* demonstrated less efficiency in the reduction of the concentrations of the heavy metals lead, cadmium and copper by 16-19% within 24 hours of treatment.

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