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Exploring Fungal Biodiversity for Lead Tolerance and Efficient Bioremediation of Pb–Contaminated Wastewater

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ABSTRACT

Heavy metal pollution, particularly by lead (Pb), poses significant environmental and public health risks. This study investigated the Pb tolerance and removal potential of a fungus isolated from the wastewater of a Common Effluent Treatment Plant (CETP), Unnao, India. The isolate demonstrated remarkable Pb tolerance, sustaining growth at concentrations up to 1200 mg/L. Pb removal experiments were conducted in potato dextrose broth under varying pH (5–9) and temperature (20–40 °C) conditions. Maximum Pb removal efficiency (>97%) was achieved at pH 6 and 28 °C, correlating strongly with optimal fungal biomass production. Removal efficiency decreased at higher Pb concentrations, pH, and temperatures, highlighting the influence of environmental factors on biosorption. The study suggests that the fungal isolate effectively binds Pb through cell wall functional groups, and its high tolerance and biosorption capacity make it a promising candidate for bioremediation of Pb-contaminated wastewater. These findings provide insights into optimizing fungal-mediated heavy metal removal under controlled environmental conditions.

Keywords: *Fungus, Pb , Biosorption, Heavy metals, Removal.*

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1. Introduction

Heavy metal contamination has emerged as a serious environmental issue due to rapid industrialization, urban expansion, and unsustainable waste disposal practices. Metals like lead (Pb), cadmium (Cd), and chromium (Cr) are persistent, non-biodegradable, and poisonous even at low levels, posing serious hazards to ecosystems and human health [1]. Pb is one of them that is commonly known to be a dangerous pollutant. It is mostly discharged through industrial effluents from the tannery, battery, metal plating, paint, and dyeing industries. Pb exposure can cause neurological diseases, kidney malfunction, poor growth in children, carcinogenic effects, and harm to the central nervous system, making its removal from wastewater essential [2]. Conventional physicochemical methods such as chemical precipitation, ion exchange, membrane filtration, and adsorption using activated carbon have been widely applied for Pb removal [3; 4]. However, these treatments are costly, operationally complex, energy-intensive, and produce hazardous sludge that requires further treatment, limiting their large-scale applicability [5]. As a result, there is a growing demand for sustainable, cost-effective, and ecologically friendly solutions. In recent years, biosorption employing microorganisms has gained popularity as a promising alternative because of its low cost, environmental friendliness, excellent selectivity, and efficiency even at low metal levels [6; 7]. Filamentous fungi are particularly suitable for bioremediation due to their rapid growth, resistance to metal stress, high surface-to-volume ratio, tolerance to pH fluctuations, and abundance of metal-binding functional groups (e.g., carboxyl, amino, phosphate, and hydroxyl groups) on their cell walls [1;8; 9]). Fungal species such as *Aspergillus niger [4]*, *A. fumigatus*, and *A. flavus [10]*, *Penicillium chrysogenum [11]*, *Rhizopus arrhizus [12]*, *Trichoderma harzianum [13]*, and *Fusarium solani [14]* have shown remarkable Pb biosorption efficiency due to their robust metabolism and diverse surface biomolecules capable of binding metal ions via chelation, ion exchange, complexation, micro-precipitation, and bioaccumulation mechanisms [15; 16]. Fungal biomass, whether live, dead, or immobilized, demonstrates biosorption characteristics that do not require nutritional supplementation, increasing its industrial feasibility [17]. Given the growing concern about Pb toxicity and the need for long-term remediation methods, this study investigates the Pb removal potential of Fungal species isolated from tannery wastewater, with a focus on removal efficiency in optimized conditions.

2. Material and Method

2.1 Isolation of fungi

Wastewater samples were collected from the Common Effluent Treatment Plant (CETP) located in Unnao (26°28′59.6″ N, 80°27′32.1″ E) in sterile bottles. The samples were brought to the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, and stored at 4 °C before analysis.

Fungal isolation was carried out by serial dilution, with 0.1 mL of the diluted wastewater inoculated onto potato dextrose agar (PDA) plates (HiMedia, India) and incubated at 28 ± 1 °C for 144 h. One of the fungal isolates was selected for subsequent experimental studies.

2.2 Pb tolerance assay

The lead tolerance of the isolate was assessed using lead nitrate ($Pb(NO_3)_2$) (Loba-Chemie, India) at concentrations of 0, 50, 100, 200, 400, 600, 800, 1000, and 1200 mg/L. For this, a 0.5 cm mycelial disc obtained from a seven-day-old culture was aseptically placed at the center of potato dextrose agar (PDA) plates (90 mm) supplemented with the respective Pb concentrations. The inoculated plates were incubated at 28 ± 1 °C for seven days. Following incubation, the metal tolerance index was determined by Eq. (1)

Metal tolerance index Ti = T/C (1)

2.3 Pb Removal at optimized pH and temperature

The Pb removal experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of potato dextrose broth (PDB) supplemented with Pb at concentrations ranging from 25 to 200 mg/L. Each flask was inoculated using a 5 mm mycelial disc from a seven-day-old fungal culture and incubated under agitation (80 rpm). Optimization studies were performed by varying pH (5–9) and temperature (20–40 °C). All treatments were carried out in triplicate. After incubation, fungal biomass was collected on pre-weighed Whatman No. 1 filter paper, dried at 60 °C for 6 h, and weighed to determine biomass yield (g/L). The dried biomass was digested using an HNO_3: H_2SO_4 mixture (3:1, v/v), and metal concentration was analyzed using an atomic absorption spectrophotometer (AA240FS). The percentage removal calculated using Eq. (2) [18].

$$\frac{Ci-Cf}{Ci} \times 100 \quad (2)$$

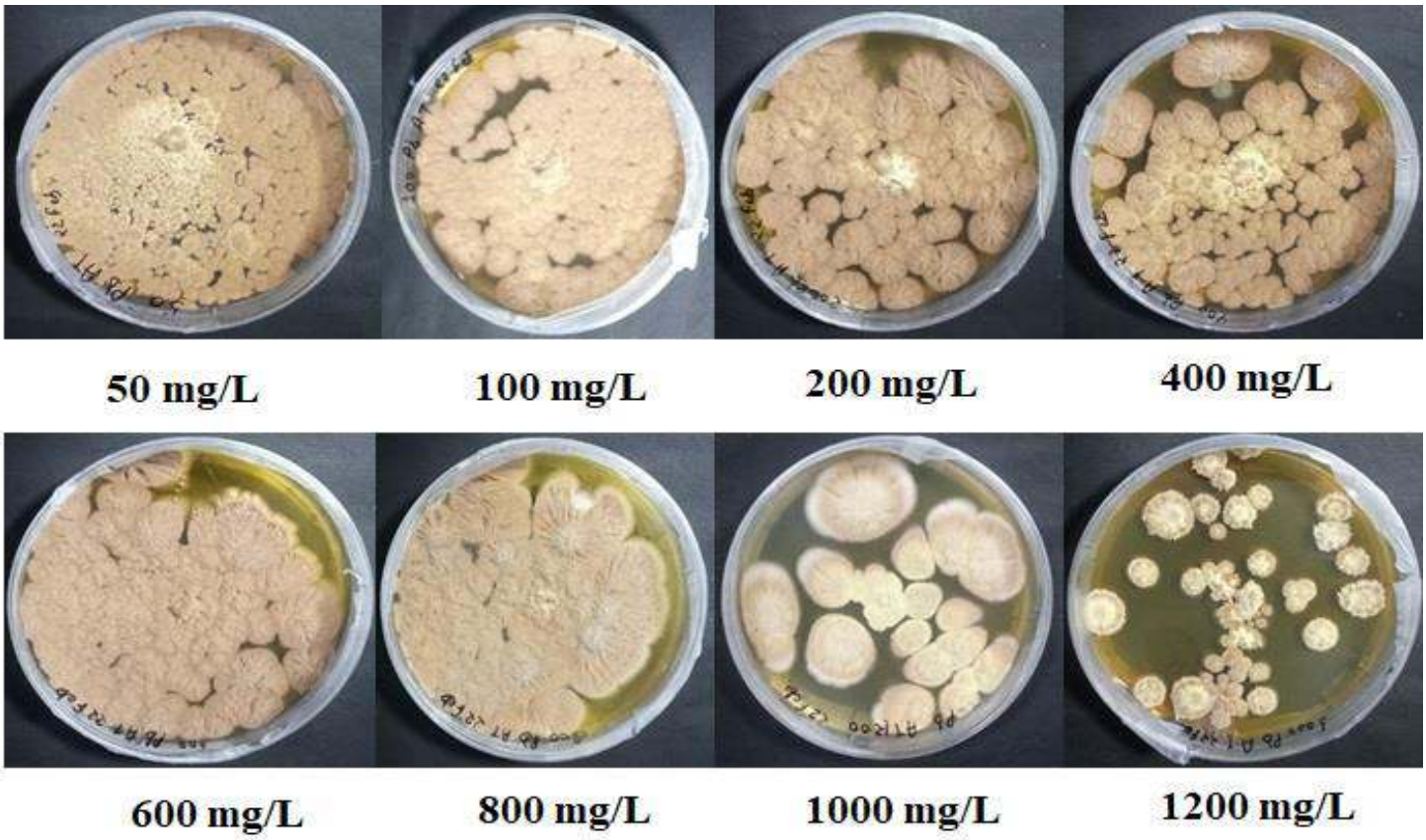

Fig: 1 Growth of isolated fungus at different concentrations of Pb

Table.1 Pb tolerance of isolated fungus

Concentration	Pb tolerance
50	1.00±0
100	1.00±0
200	0.94±0.04
400	0.89±0.04
600	0.88±0.04
800	0.67±0.07
1000	0.67±0.04
1200	0.52±0.08

3.2 Effect of optimized pH on Pb removal and biomass production

Pb removal by the fungal isolate was strongly influenced by pH, with optimal performance observed under slightly acidic conditions. Maximum Pb removal occurred at pH 6, whereas a progressive decline was noted with increasing concentration and pH, leading to negligible removal at pH 9. This trend was closely correlated with biomass production, as fungal growth was highest at a pH of 6, indicating favorable physiological conditions for metal uptake. The higher biomass observed at this pH may have enhanced the exposure and ionization of cell wall functional groups such as carboxyl, hydroxyl, and amino groups, thereby facilitating more effective Pb biosorption. In contrast, reduced biomass formation at higher Pb concentrations and alkaline pH limited metal–biomass interactions, resulting in lower Pb removal efficiency, Fig. 1(a, b). Comparable findings have been reported in previous studies, as in a study 97% removal of Pb was achieved at pH 5.0 with a biomass generation of 1.0 g/mL and 150 mg/L Pb [22], whereas in another study maximum Pb^{2+} biosorption by *Rhizopus arrhizus* at pH 4.0 and 25 °C, with a capacity of 0.501 mol kg^{-1} was observed [23]. Additionally, phosphate-solubilizing fungi including *Aspergillus niger* demonstrate notable Pb tolerance and remediation potential under controlled pH conditions, supporting the importance of optimized environmental parameters for effective biosorption [24].

Studies of multi-metal tolerant *Aspergillus* species further confirm that optimal pH plays a critical role in fungal biosorption performance across different metal pollutants [25]. Collectively, these findings confirm that optimized pH conditions are critical for maximizing fungal-mediated Pb uptake, although minor variations in optimal pH may occur among different fungal species and isolates [10].

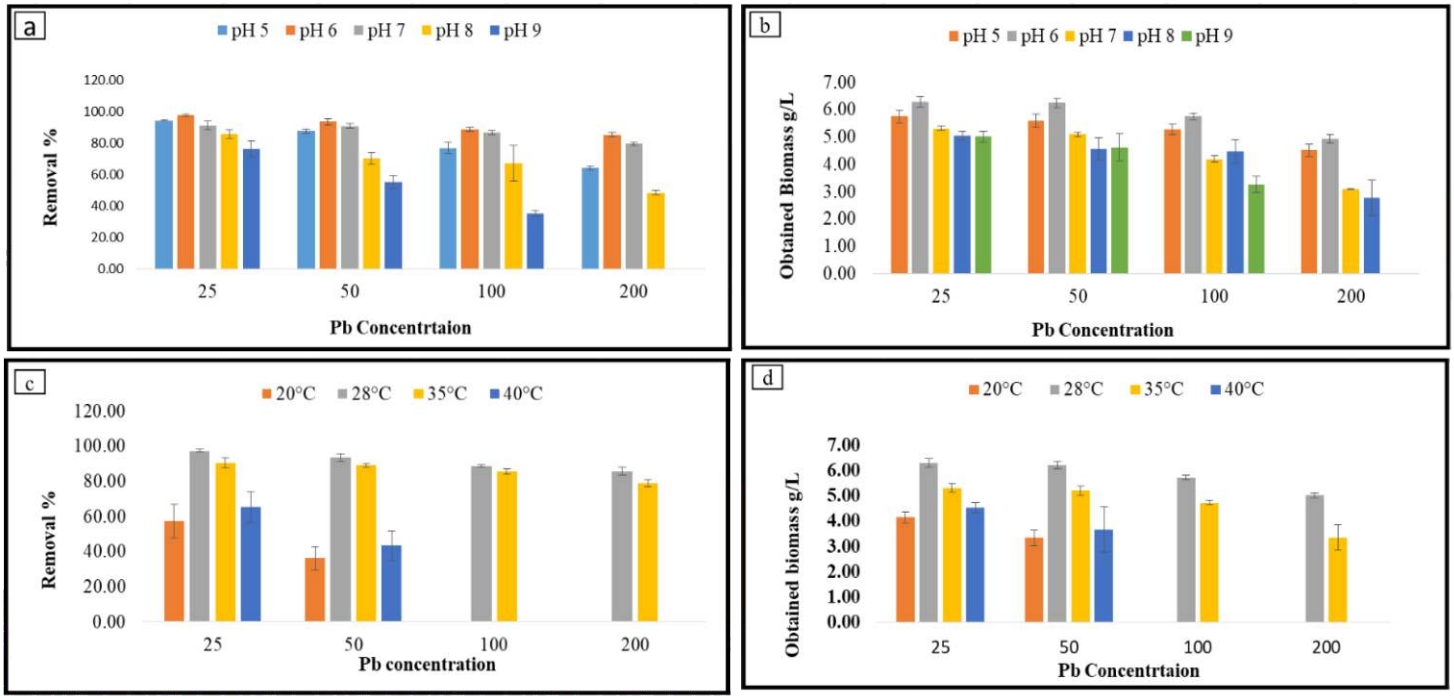

Fig.1 Effect of pH (a), temperature (c), and obtained biomass at different pH (b) and temperature (d) of fungal isolate(mean ± SD, n = 3).

3.3 Effect of optimized temperature on Pb removal and biomass production

Pb removal by the fungal isolate was also strongly affected by incubation temperature, showing a clear association between removal efficiency and biomass production. In the present study, maximum Pb removal efficiency was recorded at 28 °C, where removal exceeded 97% at lower Pb concentrations and remained high across the tested range. This temperature also supported the highest fungal biomass production indicating optimal metabolic activity for effective biosorption. At 35 °C, both Pb removal and biomass production were somewhat reduced but still substantial, suggesting partial thermal tolerance of the isolate. In contrast, temperatures below or above this optimum (20 °C and 40 °C), markedly inhibited fungal growth, resulting in reduced biomass generation and minimal Pb removal, highlighting that active fungal growth is essential for efficient biosorption. Similar results have been observed with other fungi, where biosorption efficiency typically peaks near moderate, mesophilic temperatures (25–30 °C) and declines at higher temperatures due to reduced metabolic activity and possible enzyme denaturation. For instance, *Paecilomyces lilacinus* showed maximal adsorption of various heavy metals in the 25–30 °C range, with decreased removal above 30 °C as metabolic processes were negatively affected [26]. Additionally, fungal Pb biosorption studies often report optimal removal near ambient temperatures, such as ~25 °C for *Panaeolus papilionaceus* Pb uptake, further, supporting the importance of moderate temperature for effective fungal bioremediation [27]. *Aspergillus aculeatus* [28] and *Aspergillus flavus* [29], highlighting that moderate incubation temperatures favor active fungal physiology and enhance metal removal performance.

All these findings indicate that Pb removal efficiency is closely linked to temperature-driven changes in fungal growth and metabolism, emphasizing that conditions supporting active fungal development are essential for effective and practical bioremediation. Fig. 1©, d).

4. Conclusion

This study was conducted under optimized experimental conditions, where the effects of varying pH levels and incubation temperatures were systematically evaluated. The fungal isolate exhibited high resistant to Pb, sustaining growth at doses of up to 1200 mg/L and rapidly removing Pb from aqueous solutions. Maximum removal (>97%) was reached at pH 6 and 28 °C, which coincided with peak biomass production, emphasizing the importance of fungal growth in biosorption. Experimental parameters such as pH, temperature, and metal content have a significant impact on removal efficiency. This study is distinguished by the use of a naturally acclimated fungal isolate from tannery wastewater, which shows higher lead tolerance and biosorption capability when compared to previous studies. Future research could explore large-scale applications, simultaneous removal of multiple heavy metals, and the recycling of fungal biomass to establish efficient, practical, and sustainable bioremediation strategies.

Credit authorship contribution statement

Priyanka: Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing, Formal analysis, Validation. Shiv Kumar Dwivedi: Conceptualization, editing, Validation, Supervision.

Declaration of Competing Interest

The authors declare there is no possible conflict of interest in the research, authorship, or publication of this paper.

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