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Bioremediation Potential of the Endophytic Fungus *Penicillium janthinellum* for Heavy Metal Removal from Wastewater

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AbstractContamination of aquatic ecosystems with heavy metals poses a significant environmental and public health risk because of the persistent nature, bioaccumulative potential, and toxic effects of metals such as Pb, Cd, Cu, Zn, and Cr.. The present study evaluates the bioremediation potential of the endophytic fungus *Penicillium janthinellum* isolated from the stem tissues of *Zygophyllum coccineum*. The isolate was morphologically and molecularly identified using ITS rDNA sequencing and screened for its biosorption capacity toward heavy metals in wastewater collected from drains south of Manzala Lake, Egypt. Spore suspensions were immobilized in alginate beads and applied in batch systems to assess the effects of pH, temperature, and contact time on metal removal. Results revealed that *P. janthinellum* exhibited high biosorption efficiency, with removal percentages ranging from 67–74% for Pb, Cu, Zn, Cd, and Cr, respectively. Optimal removal occurred at slightly acidic pH (5–6), moderate temperature (30–35 °C), and contact time of 200–300 min. The fungus demonstrated strong tolerance to elevated metal concentrations and maintained stable performance under natural wastewater conditions. These findings highlight the potential of *P. janthinellum* as an eco-friendly and cost-effective biosorbent for the remediation of heavy metal–polluted effluents.

keywords: *Penicillium;* Biosorption; Heavy metals; Wastewater*;* Optimization.

Introduction

Heavy metal contamination of wastewater is greatest environmental concern due to the stability, bioaccumulation, and toxicity of the pollutants. Industrial wastewater discharges from mining, metal finishing, and electroplating introduce cadmium (Cd), lead (Pb), copper (Cu), chromium (Cr), and other metals into the aquatic environment, which pose great danger to human health and the ecosystem [1,2]. Conventional treatment methods like chemical precipitation, ion exchange, and membrane filtration are typically energy-consuming, inefficient at low concentrations, and generate secondary waste [3,4].

Microbial bioremediation has been proposed to be a cost-effective and environmentally sound alternative. Fungi, in particular, have been of particular interest since they are resistant to extreme conditions, have a very large surface area, and include functional groups in their cell walls that can chelate metal ions [5,6]. Fungal biosorption mechanisms include passive adsorption to cell walls, active uptake, and enzymatic conversion of metals [7].

The *Penicillium* genus is widely studied for heavy metal biosorption worldwide. *P. chrysogenum* and *P. simplicissimum* are some of the species that have been found to exhibit heavy removal capacities of Pb(II) and Cd(II) [8,9]. Among these, *Penicillium janthinellum* has been a promising one since it is tolerant and has high biosorption potential. Gong et al. [10] reported that *P. janthinellum* eliminated more than 90% of Pb(II) under batch conditions. Zhao et al. [11] also showed that polyvinyl alcohol–sodium alginate beads immobilized *P. janthinellum* with efficient removal of Pb(II), Cd(II), and Cu(II). Zhao et al. [12] later demonstrated that co-immobilization of *P. janthinellum* with graphene oxide enhanced the removal efficacy, where the removal of more than 85% of Pb(II), Cu(II), and Fe(III) in the mixed systems. Genomic study also revealed the presence of metal transport and detoxification genes, testifying further to the remediation potential of this fungus [13].

Despite these promising results, most of the researches were carried out on immobilized or inactive biomass, while fewer researches examined systematically the operation of live *P. janthinellum* under pure batch operation. Batch experiments are fundamental to biosorption performance, such as kinetics, isotherms, and operational conditions like pH, temperature, contact time, and dosage of biomass [14]. Hence, the present study proposes to assess the bioremediation capability of *Penicillium janthinellum* for heavy metal (Pb, Cd, Cu, Zn, and Cr) removal from wastewater in batch conditions. Optimization of experimental parameters is anticipated to give useful information towards using the fungus in sustainable wastewater treatment.

2. Materials and Methods

2.1. Isolation and Purification of Endophytic Fungi

Healthy mature plants of *Zygophyllum coccineum* were collected from a coastal habitat, placed in sterile bags, and transported to the laboratory. stem was washed with running tap water and cut into ~5 mm segments. Surface sterilization was performed using 70% ethanol (1 min), 2% sodium hypochlorite (3 min), and 70% ethanol (30 s), followed by two washes in sterile distilled water. Sterilized portions (three for each organ) were inoculated on PDA medium with 250 mg/L chloramphenicol, five replicates of each organ. Plates were incubated at 26 ± 2 °C [15]. The outgrowth hyphae were plated on new PDA plates after seven days for purification. Isolates were stored in PDA slants, subculture monthly, incubated for 5 days at 28 °C, and stored at 4 °C.

2.2. Biomass and Spore Production

Actively growing PDA cultures (28 °C, 5–7 days) were used as inoculum. Mycelial plugs (5 mm) were transferred to 500 mL Erlenmeyer flasks containing 200 mL potato dextrose broth and incubated statically at 28 ± 2 °C for 10–14 days [16]. Biomass was harvested by filtration, washed with sterile distilled water, and used either fresh or dried at 60 °C. Spore suspensions were prepared from 7–10-day-old cultures of PDA by flooding with sterile 0.85% saline + 0.01% Tween 80, scraping, and filtering through sterile cotton. Spore concentration was adjusted to ~1×10^6 spores·mL^{-1} using a hemocytometer [17,18].

2.3. Molecular Identification

One representative fungal isolate was determined by internal transcribed spacer (ITS) sequence analysis. ITS rDNA region was amplified with the primers ITS1-F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). PCR reactions (25 µL) contained 1× PCR buffer, 0.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.5 µL of each primer, 2.5 U Taq DNA polymerase (QIAGEN, USA), and 1 µg genomic DNA. Amplification was performed in a DNA Engine Thermal Cycler (PTC-200, BIO-RAD, USA) according to the following protocol: initial denaturation at 94 °C for 3 min; 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min; final extension at 72 °C for 10 min. PCR amplicons were analyzed on 1% agarose gel and were commercially sequenced (Sigma Co., Egypt). Sequences were blasted by using the NCBI BLAST tool against GenBank database. Multiple sequence alignment was performed with Clustal Omega, and phylogenetic trees were constructed by the neighbor-joining method in MEGA v6.1 with 1000 bootstrap replicates.

2.4. Batch screening experiments

Fungal isolate was screened for their potential as biosorbents of Pb, Cd, Cu, Zn, and Cr from wastewater samples collected from drains south of Manzala Lake. Preliminary MIC assays confirmed their resistance to high metal concentrations [18]. Resistant isolate was grown on PDA, and spore suspensions were immobilized in alginate beads, pretreated with 0.1 M HCl. Beads were enclosed in cellulose tissue (“tea-bag” format, 4 × 4 cm) and suspended in 250 mL glass beakers containing heavy metal solutions [19,20]. Empty alginate beads served as blanks to confirm that biosorption was biomass-dependent. Screening was conducted using natural wastewater samples from three drains with predetermined initial metal concentrations.

Following batch incubation, residual metal concentrations were determined by atomic absorption spectroscopy, and removal efficiency was calculated as:

$$\textbf{Biosorption removal Rate } \% = \frac{C_i - C_f}{C_i} * 100$$

where C_i is the initial concentration (ppm) and C_f is the final concentration (ppm).

3. Results and Discussion

3.1. Isolation and Purification of Endophytic Fungi

In this study, an endophytic fungus was isolated from the stem of the xerophytic plant *Zygophyllum coccineum*, collected from the northern sector of the Nile Delta, Egypt (Figure 1). The fungal isolates were obtained after surface sterilization of the stem segments and incubation on suitable nutrient media. The fungal colonies appeared gradually with distinct morphological characteristics compared to epiphytic fungi (Figure 1). Purification was achieved through repeated sub-culturing to obtain a single pure isolate representing the true endophytic fungus associated with the plant tissue. The successful isolation and purification process indicates the ability of this desert plant to harbor endophytic fungi well adapted to harsh environmental conditions, highlighting its potential as a promising source of bioactive compounds with significant biological activities.

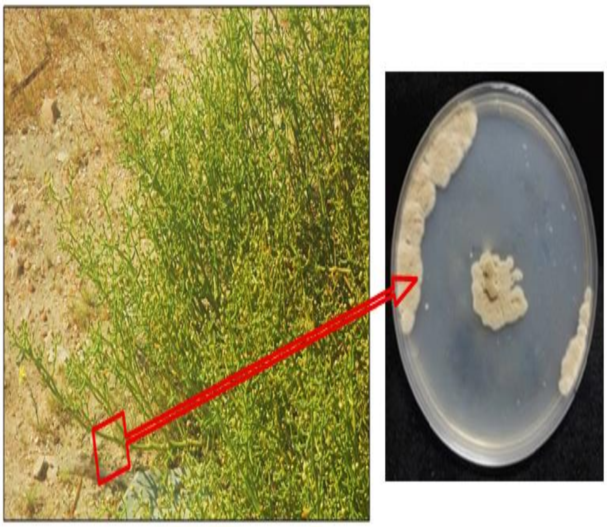

Figure 1. General view of *Zygophyllum coccineum* and pure culture of isolated fungi.

Morphologically, *P. janthinellum* exhibited typical characteristics of the genus *Penicillium*. The colonies were filamentous, fast-growing, and convex with curled or undulated margins (Figure 1). The surface pigmentation appeared yellowish-brown, while the reverse side displayed a darker brown coloration. These observations are consistent with recent descriptions by Wang et al. [21], who reported that *P. janthinellum* typically forms green to blue-green or colorless conidial masses, with mycelia that may appear pale pink, yellowish, or white and reverse colony pigmentation ranging from reddish-brown to olive-brown. Such morphological traits confirm the taxonomic identity of the isolated strain and reflect its adaptability to the endophytic habitat within the stem tissues of *Z. coccineum*.

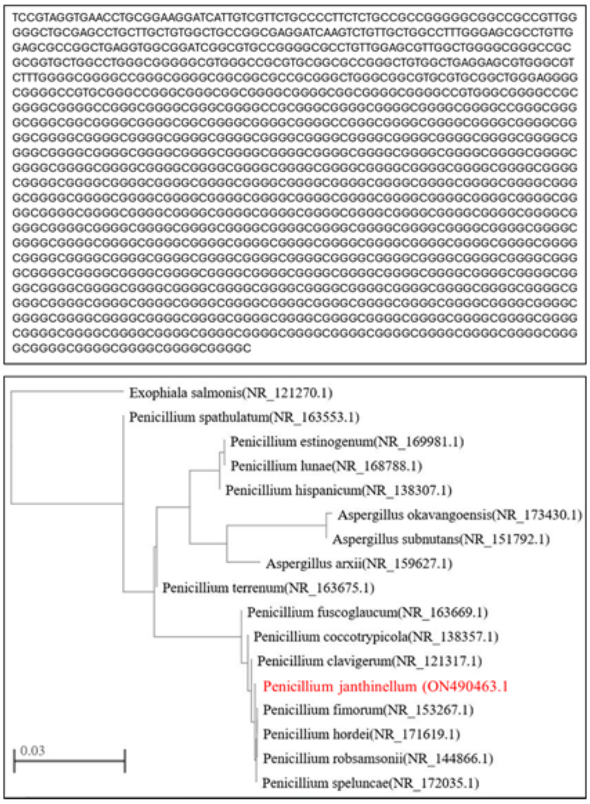

Figure 2. The phylogenetic tree derived from 16S rRNA gene sequences of *Penicillium janthinellum*.

3.2. Molecular Identification

For molecular identification, genomic DNA was extracted from the purified fungal isolate obtained from the stem tissues of *Z. coccineum*. The internal transcribed spacer (ITS) region of ribosomal DNA was amplified using universal primers ITS1 and ITS4. The PCR product was purified and sequenced, and the resulting sequence was compared with available sequences in the NCBI GenBank database using the BLAST program. The isolate showed a high similarity percentage (≥99%) with *Penicillium janthinellum* sequences, confirming its identity at the species level. The obtained sequence was deposited in GenBank under a specific accession number. Phylogenetic analysis based on ITS sequences clustered the isolate with reference strains of *P. janthinellum*, supporting its accurate molecular identification (Figure 2). These results are consistent with previous reports emphasizing that ITS rDNA sequencing is a reliable and widely accepted molecular marker for distinguishing *Penicillium* species [21,22].

3.3. Batch screening experiments

3.3.1. Heavy metal removal by Penicillium janthinellum

Table 1 indicates that *P. janthinellum* exhibited a high and consistent ability to remove heavy metals from drain water. Lead (Pb) removal ranged between 68.23% and 70.73% across sites, with a mean of 69.33 ± 0.43% and very low variability (CV = 1.84%), reflecting excellent stability. Cadmium showed a similar trend, with removal efficiencies between 66.36–69.68%, averaging 68.07 ± 0.56% and low variability (CV = 2.45%). Copper removal was higher, from 66.55% at El-Serw to 74.46% at Hadous, with a mean of 71.46 ± 1.43% and moderate consistency (CV = 6.00%) (Table 1). Zinc removal ranged from 65.75–71.87%, averaging 68.52 ± 1.03% with low variability (CV = 4.53%), confirming uniform biosorption capacity. For Cr, performance was somewhat variable, with removal efficiencies spanning 61.07–73.88% and a mean of 67.82 ± 2.15%, though variability remained within a low range (CV = 9.49%) (Table 1).

Similar trends have been reported for other *Penicillium* species; for example, *P. chrysogenum* and *P. citrinum* have been shown to remove up to 70% of Pb and 65–68% of Cd from contaminated waters [23]. Moreover, Wang et al. [21] reported that *P. janthinellum* exhibited strong biosorption of Pb and Cu in industrial effluents, with efficiencies exceeding 70%, highlighting the genus's affinity for heavy metals through cell wall functional groups such as carboxyl, hydroxyl, and amino moieties. The consistency of these findings underlines the robustness of *P. janthinellum* for biosorption in complex matrices, including real wastewater containing competing ions and organic matter, which often reduce adsorption efficiency compared to synthetic solutions. Additionally, previous studies noted some variability in Cr removal due to differences in speciation and environmental conditions, which aligns with the moderate variation observed in our Cr biosorption data (CV ~9.5%). Overall, these results corroborate the potential of *P. janthinellum* as an eco-friendly biosorbent for heavy metal remediation, supporting its practical applicability in wastewater treatment.

Table 1. Heavy metal removal from drain water samples by *Penicillium janthinellum*.

Parameter	Drain	Ci (µg/L)	Cf (µg/L)	Removal %	Mean ± SE	CV
Pb	El-Serw	6.62	2.10	68.23	69.33±0.43	1.84
	Faraskur	9.57	2.80	70.73		
	Hadous	4.14	1.28	69.03		
Cd	El-Serw	11.85	3.77	68.18	68.07±0.56	2.45
	Faraskur	15.32	5.15	66.36		
	Hadous	6.95	2.11	69.68		
Cu	El-Serw	3.51	1.17	66.55	71.46±1.43	6.00
	Faraskur	4.68	1.25	73.38		
	Hadous	2.89	0.74	74.46		
Zn	El-Serw	8.05	2.76	65.75	68.52±1.03	4.53
	Faraskur	11.05	3.11	71.87		
	Hadous	7.15	2.29	67.93		
Cr	El-Serw	18.91	5.95	68.52	67.82±2.15	9.49
	Faraskur	24.64	6.44	73.88		
	Hadous	14.98	5.83	61.07		

CV: Coefficient of Variation; CV% < 10%: Low variability (results are very consistent); 10% ≤ CV% < 20%: Moderate variability; 20% ≤ CV% < 30%: High variability; CV% ≥ 30%: Very high variability / not consistent.

3.3.3. Optimization of Biosorption Parameters

The biosorption efficiency of *P. janthinellum* toward heavy metals in drain water was markedly affected by the pH of the medium (Figure 3). At acidic conditions (pH 3–4.5), removal percentages were moderate, ranging between 48–63%, with Pb and Cu exhibiting the highest adsorption efficiencies (63% and 67%, respectively). Increasing the pH to 5–6 resulted in a significant improvement in metal removal, where Pb, Cd, Cu, Zn, and Cr reached maximum efficiencies of 80%, 73%, 82%, 75%, and 77%, respectively. This enhancement can be attributed to decreased competition between hydrogen ions and metal cations for active binding sites on the fungal surface. However, at alkaline pH (7–8), the biosorption efficiency declined, particularly for Pb, Cd, Zn, and Cr, while Cu maintained relatively high adsorption (70–79%). Overall, the optimal pH range for maximum biosorption was 5–6, indicating favorable ionization and charge distribution on both the metal ions and the fungal cell wall [24,25,26].

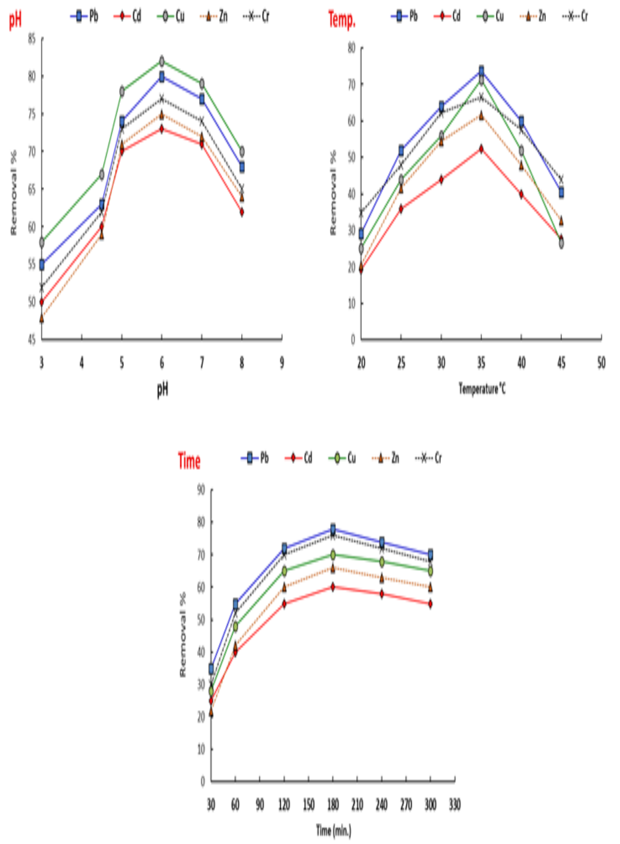

Figure 3. The effect of pH, temperature and time on the removal efficiency of heavy metals by *Penicillium janthinellum*.

Temperature variation had a pronounced impact on the biosorption performance of *P. janthinellum* (Figure 3). At lower temperatures (20–25 °C), removal efficiencies were relatively low (19–52%), suggesting limited metabolic and physicochemical interactions between the biomass and metal ions. As the temperature increased, metal removal improved, reaching a maximum at 35 °C, where Pb (74%), Cu (71%), Zn (62%), Cd (52%), and Cr (67%) exhibited their highest biosorption rates. This enhancement reflects the increased mobility of metal ions and improved diffusion onto the fungal cell surface. However, further elevation to 40–45 °C resulted in a noticeable decline in removal efficiency, possibly due to thermal denaturation of cell wall components or desorption of previously bound ions. These findings indicate that *P. janthinellum* exhibits optimal biosorption performance within the temperature range of 30–35 °C [27,28].

The contact time between fungal biomass and metal ions significantly influenced biosorption capacity (Figure 3). During the early stages (30–60 min), metal uptake was relatively modest (22–55%) due to the progressive occupation of available binding sites. A substantial increase was observed after 120–180 min, with maximum removal recorded for Pb (78%), Cr (76%), Cu (70%), Zn (66%), and Cd (60%). This phase represents the establishment of biosorption equilibrium, where most active sites are saturated. Extending the contact time beyond 240–300 min did not enhance metal removal; in some cases, a slight decline was observed, likely due to desorption or redistribution of metal ions. Thus, the optimal contact time for effective biosorption by *P. janthinellum* was found to be between 120 and 180 minutes, after which equilibrium was achieved [29,30,31].

The plots demonstrate that (Figure 4) optimal biosorption of heavy metals by *P. janthinellum* occurs under slightly acidic pH (5–6), moderate temperature (30–35 °C), and prolonged contact time (200–300 min). The results confirm that biosorption efficiency is governed by the interplay of physicochemical parameters affecting the availability of binding sites and the mobility of metal ions.

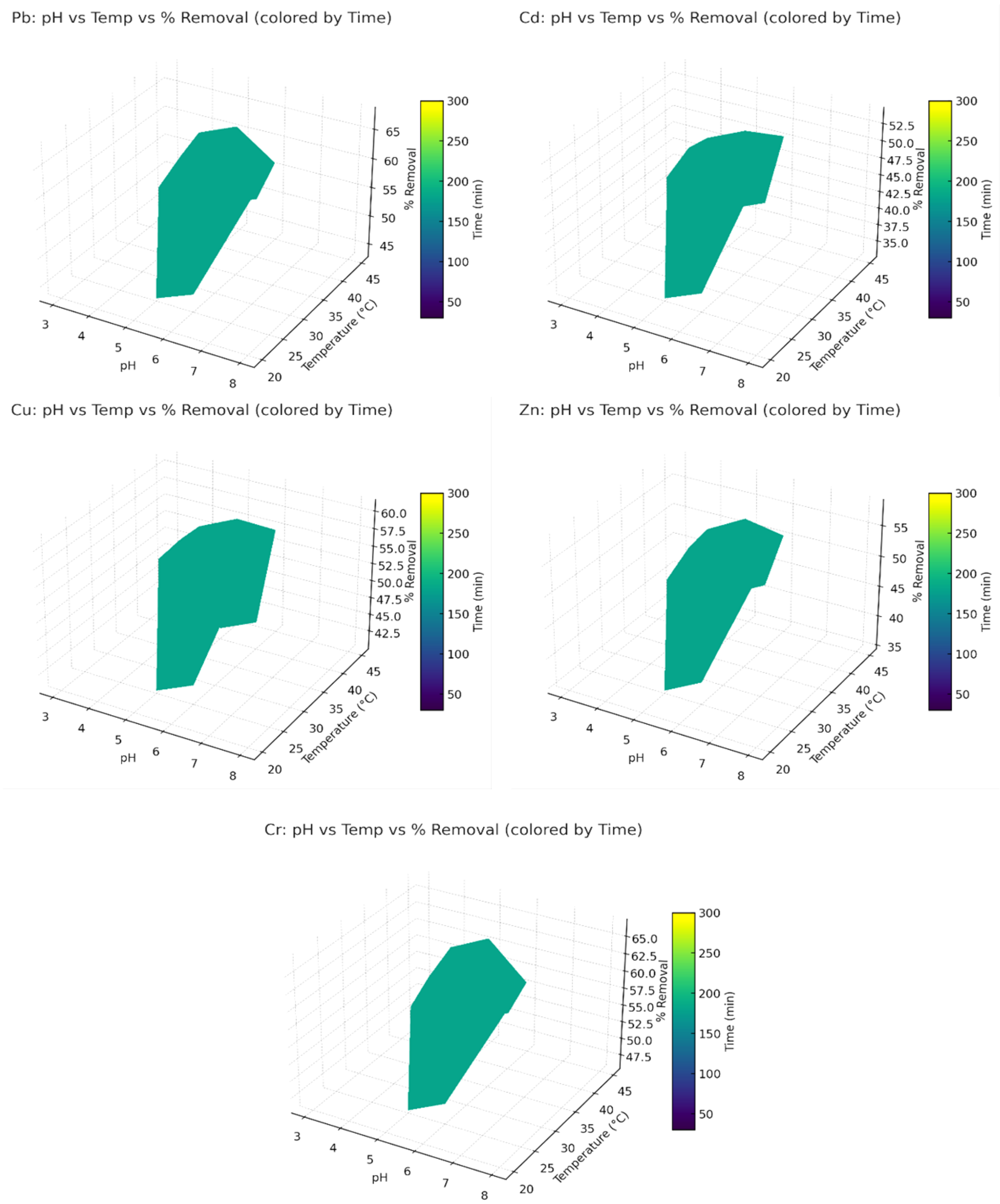

Figure 4. Three-Dimensional Response Surface Plots showing the interactive effects of pH, temperature, and contact time on the biosorption efficiency of Pb, Cd, Cu, Zn, and Cr by *Penicillium janthinellum.*

4. Conclusion

The present study demonstrated that the endophytic fungus *P. janthinellum* possesses remarkable potential for the bioremediation of heavy metal–contaminated wastewater. The fungus effectively removed Pb, Cd, Cu, Zn, and Cr, with optimal biosorption observed at pH 5–6, temperature 30–35 °C, and contact time of 200–300 min. Its high tolerance to metal stress and consistent performance in real wastewater highlight its robustness and environmental adaptability. The findings confirm that *P. janthinellum* can serve as a sustainable, low-cost biosorbent for heavy metal removal, offering a promising alternative to conventional chemical treatment methods in wastewater purification.

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