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Bioleaching and biosorption of Cu^{2+} , Pb^{2+} , and Cd^{2+} from laptop PCBs by effective fungal strains

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ABSTRACT

The toxic heavy metals in waste printed circuit boards (PCBs) in electrical and electronic devices make them particularly challenging to manage. This research emphasized the bioleaching and biosorption potential of two effective fungal strains for removing Cu^{2+} , Pb^{2+} , and Cd^{2+} from laptop PCBs. The bioleaching experiment demonstrated that the two-step bioleaching process using *Aspergillus niger* effectively extracted heavy metal ions from laptop PCBs. One kilogram of laptop PCB samples contained 130.25 ± 1.53 g of Cu, 18.81 ± 0.09 g of Pb, and 0.037 ± 0.004 g of Cd. The results suggest that Cu^{2+} , Pb^{2+} , and Cd^{2+} achieved maximum bioleaching efficiencies of 73.06%, 63.91%, and 70.53%, respectively, after a 21-day incubation period. The biosorption experiment using immobilized *Humicola phialophoroides* biomass pretreated with NaOH demonstrated higher efficiency in removing heavy metal ions from leachate than non-immobilized biomass in bioremediation processes. The maximum heavy metal biosorption by the immobilized cells was achieved at a solution at 30 °C after an equilibrium time of 120 minutes at pH 7. The heavy metal ion biosorption capacities were 184.10 ± 3.65 mg Cu g⁻¹ dry wt., 39.60 ± 1.21 mg Pb g⁻¹ dry wt., and 2.28 ± 0.04 mg Cd g⁻¹ dry wt. The temperature had little effect, while the pH value significantly influenced the sorption process.

1. Introduction

Electronic waste (e-waste) is one of the world's largest and fastest-growing waste streams. The world produced 62,000 tonnes of e-waste in 2020, but only 13,800 tonnes (22.3%) were officially documented as collected and recycled. Statistics show that approximately 216 million laptops were shipped in 2022 [1]. Laptops are typically designed

with an average lifespan of three to five years, suggesting a substantial increase in laptop waste is anticipated shortly. In 2022, Thailand was the second-largest generator of e-waste in Southeast Asia, producing 753 million kg, with a per capita rate of 10.5 kg [2]. Thai households are the main contributors to e-waste, with most of it collected and processed by the informal sector [3]. Non-standard methods of dismantling and recycling

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e-waste are major contributors to heavy metal contamination in the environment, leading to serious environmental pollution, and may be lethal to humans. [4]. PCBs are a significant part of e-waste and contain many of these hazardous materials. PCBs are the internal components that carry electrical signals within electronic devices. The solder material used to create conductive bonds between components and the PCB surface, along with components embedded in PCBs like batteries, may contain toxic heavy metals: lithium (Li), lead (Pb), arsenic (As), mercury (Hg), cadmium (Cd), etc.

The biomining concept is currently defined as a two-stage integrated biological system. The first stage involves bioleaching, and the second stage focuses on biosorption, enabling heavy metal removal and recovery from e-waste. Some species of heterotrophic microorganisms, such as *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., etc., are commonly used to extract heavy metals from e-waste [5]. Among them, *Aspergillus niger* is generally regarded as a low-risk microorganism (Risk Group 1) and has been extensively used in industrial fermentation processes. Moreover, previous studies have demonstrated its highest potential for heavy metal leaching PCBs [6–8]. Several microorganisms, such as bacteria, algae, yeasts, and fungi, have been investigated for their biosorption capabilities and demonstrate significant potential in precipitating heavy metals from water and wastewater [9]. Fungal microorganisms can uptake heavy metal ions from contaminated water through various mechanisms, including surface complexation, biosorption, and ion exchange [10].

Furthermore, immobilizing non-living microbial cells on synthetic polymeric or natural matrices through entrapment has been shown to significantly enhance performance, biosorption capacity, and the lifespan of biosorbents for recycling and regeneration by several orders of magnitude. Natural polymers, such as alginate, chitosan, and chitin, are commonly used as immobilization matrices due to their efficiency. Chitosan, alginate, and chitin are among the most frequently used as a matrix in biocomposites because of their high biocompatibility, low cost, and non-toxic nature [11]. Recent research

indicates that the *Humicola phialophoroides* fungus has a high removal efficiency of heavy metal ions from aqueous solutions when the fungal is pretreated with NaOH. However, most research has been limited to synthetic water spiked with a single heavy metal ion [12,13].

This research aims to evaluate the adsorption capacity of *H. phialophoroides* biomass after being pretreated with NaOH and immobilized in sodium alginate. The study focuses on decreasing mixed heavy metal ions (Cu^{2+} , Pb^{2+} , and Cd^{2+}) from laptop PCBs through the bioleaching process using *A. niger* fungus.

2. Materials and methods

2.1. Collection, preparation, and determination of Cu^{2+} , Pb^{2+} , and Cd^{2+} content in laptop PCBs

A total of ten discarded laptops were collected from 3 local recycling shops in Nakhon Sawan province, located in the lower northern region of Thailand.

The sample for this study was restricted to laptops released between 2006 and 2016. The PCB motherboards from laptops of various brands were manually dismantled to extract capacitors, resistors, processor carriers, diodes, etc. Several of these boards were first crushed using a cutting mill to obtain pieces approximately 5 cm in size. These larger pieces were then manually cut into smaller particles, achieving a size fraction of less than 2.5 mm. Deionized water was used to wash the laptop PCB samples, and they were dried in a hot air oven for 1 day at 80°C. Briefly, 1 g of the dried laptop PCB sample was digested in a 250-mL flask using 5 mL of concentrated HNO_3 and 10 mL of concentrated HCl.

The digestion mixture was heated on a hot plate inside a fume hood until the solution became clear and the PCB sample was completely dissolved. Whatman No. 45 filter paper was used to filter the digested solution and dilute it to 25 mL with deionized water. Afterward, the solution was placed in a separate polypropylene bottle for heavy metal analysis. The concentrations of Cu^{2+} , Pb^{2+} , and Cd^{2+} were measured using Atomic Absorption Spectroscopy (AAS) with the PerkinElmer PinAAcle 900T model (PerkinElmer Inc., Shelton, CT, USA), employing the flameless graphite furnace method.

2.2. Fungal cultivation and preparation

The Biology and Biotechnology program at Rajabhat Nakhon Sawan University provided filamentous fungus *A. niger* strains ATCC 1015 for this work. The fungus *H. phialophoroides* strain was isolated from the cadmium-contaminated sediment of the Mae Tao Creek in the Mae Sot District in Tak Province, Thailand [12]. Seven-day-old fungal spores were harvested from the surface of the potato dextrose agar slant. Spore numbers were counted via microscopy using a hemocytometer and adjusted to 10^7 spores mL^{-1} of spore suspension.

2.2.1. NaOH-pretreated biomass preparation

The process of NaOH-pretreated biomass preparation followed the research methodology of Netpae [13]. Spore suspension of *H. phialophoroides* was transferred to a 250 mL Erlenmeyer flask containing 50 mL potato dextrose broth medium. The medium was incubated under aerobic conditions at 30 ± 2 °C in a shaking incubator at 150 rpm for 3 days. The harvested viable biomass was immersed in 10% NaOH solution at 30 ± 2 °C for a 30-minute period. The fungal biomasses were filtered and rinsed with deionized water until the water reached neutral pH. Afterward, the harvested biomasses were inactivated by autoclaving at a temperature of 121°C under pressure at 15 psi for 30 minutes and then oven dried at 80°C for 48 hours. When the biomass was dry, it was powdered and sieved to a size less than 0.5-1.0 mm diameter. The substrates were powdered and kept until further use.

2.2.2. Immobilized cell bead preparation

The immobilized cell consisted of *H. phialophoroides* biomass pretreated with NaOH, mixed with 4% sodium alginate in a 1:38 ratio [7]. The mixture was stirred for 1 hour at 30 ± 2 °C before being dripped through a nozzle into a 4% (w/v) CaCl_2 solution, where it was gently stirred to facilitate polymerization and bead formation. The resulting cell beads with an approximate diameter of ~2 mm was hardened overnight in the same solution at 4 °C. After filtration, the collected cell beads were rinsed multiple times with deionized water and air-dried for 12 hours at room temperature for future use in the biosorption tests.

2.3. Preparation of the leachate

A two-step bioleaching process was conducted according to the protocol established by Netpae and Suckley [8]. The *A. niger* fungus was initially cultured in Richards's broth without the laptop PCBs for seven days, after which the sterilized and dried laptop PCBs samples were introduced. The leachate by *A. niger* was prepared by adding 0.3 g of the ground laptop PCBs and the fungal spore suspension with 80 mL of the broth media in a 250 mL Erlenmeyer flask and shaken at 150 rpm at room temperature (28 ± 2 °C). After 15, 18, 21, 24, and 27 days, the suspension was filtered through 45 μm filter paper. The fungal growth and metabolic activity of *A. niger* were monitored by measuring citric acid production, pH, and dry biomass weight. The filtrate was then analyzed for pH using a pH meter Seven2Go (Mettler Toledo, Greinfensee, Switzerland); the concentrations of Cu^{2+} , Pb^{2+} , and Cd^{2+} were determined using Atomic Absorption Spectroscopy. The production of citric acid by *A. niger* was analyzed using Shimadzu high-performance liquid chromatography with diode array detector (HPLC-DAD) detection at 210 nm [14]. The resulting fungal mycelia were washed three times with distilled water and oven-dried at 70°C for 24 hours before weighing.

2.4. Procedures of biosorption experiments

The efficiency of the *H. phialophoroides* biomass pretreated with NaOH and cell beads in adsorbing Cu^{2+} , Pb^{2+} , and Cd^{2+} from bioleaching was determined in batch adsorption experiments. A 45 mL volume of the leachate was initially combined with 200 mg of each adsorbent. After adjusting the conditions of the samples to pH 7 by using a 0.1 M NaOH solution, the final volume was adjusted to 50 mL with deionized water. The adsorption capacities of the two sorbents for heavy metal ions from the leachate were evaluated under the various optimized conditions of temperature, pH, and contact time for the adsorption of Cu^{2+} , Pb^{2+} , and Cd^{2+} . Before mixing with the sorbents, the leachate temperature was adjusted to 30, 40, 50, 60, and 70 °C, while the initial pH was prepared within a range of 3.0 to 7.0 (incremented at pH 3, 4, 5, 6, and 7) by using 0.1 M NaOH or 0.1 M HNO_3 . The contact time was examined over up to 180 minutes. Samples were shaken at 160 rpm and

collected at intervals of 30, 60, 90, 120, 150, and 180 minutes. The suspension was then filtered using 45 μm filter paper, and the resulting supernatants were analyzed for heavy metal concentrations by atomic absorption spectrometry. The amount of heavy metal uptake (mg g^{-1}) was obtained using the following equation:

$$q = ((C_i - C_f)V) / W \quad (1)$$

where q represents the heavy metal uptake (mg g^{-1} dry wt.), and C_i and C_f denote the initial and the equilibrium concentrations of heavy metal in the solution (mg L^{-1}), respectively. V is the volume of the heavy metal-bearing solution contacted with sorbent (mL), and W is the mass of the sorbent used (g). Figure 1 illustrates the complete research methodology employed in this study.

2.5. Statistical methods

The experimental data are reported as the average of triplicates $\pm$ standard deviation. One-way analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT) post-hoc test were carried out to indicate significant differences at $p < 0.05$.

3. Results and discussion

3.1. Heavy metal content in laptop PCBs

The high contents of copper and lead found in laptop PCBs from e-waste recycling shops were 130.25 ± 1.53 and $18.81 \pm 0.09 \text{ g kg}^{-1}$ PCBs, respectively. Among the heavy metals analyzed, cadmium was found to be the lowest at a content of $0.037 \pm 0.004 \text{ g kg}^{-1}$ PCBs.

These proportions align well with those reported in the literature: Adie et al. [15], Priya and Hait [16], and Trinh et al. [17]. This study found that the content of heavy metals was relatively high, especially for Pb^{2+} and Cd^{2+} in the laptop PCB samples compared with studies by Tunali et al. [18].

The diversity of the samples, the characterization methods employed, and the source of the e-waste complicate direct comparisons with data available in the literature.

It is significant to note that e-waste's composition can change over time due to technological advancements; also, different brands may have different heavy metal concentration levels in their products.

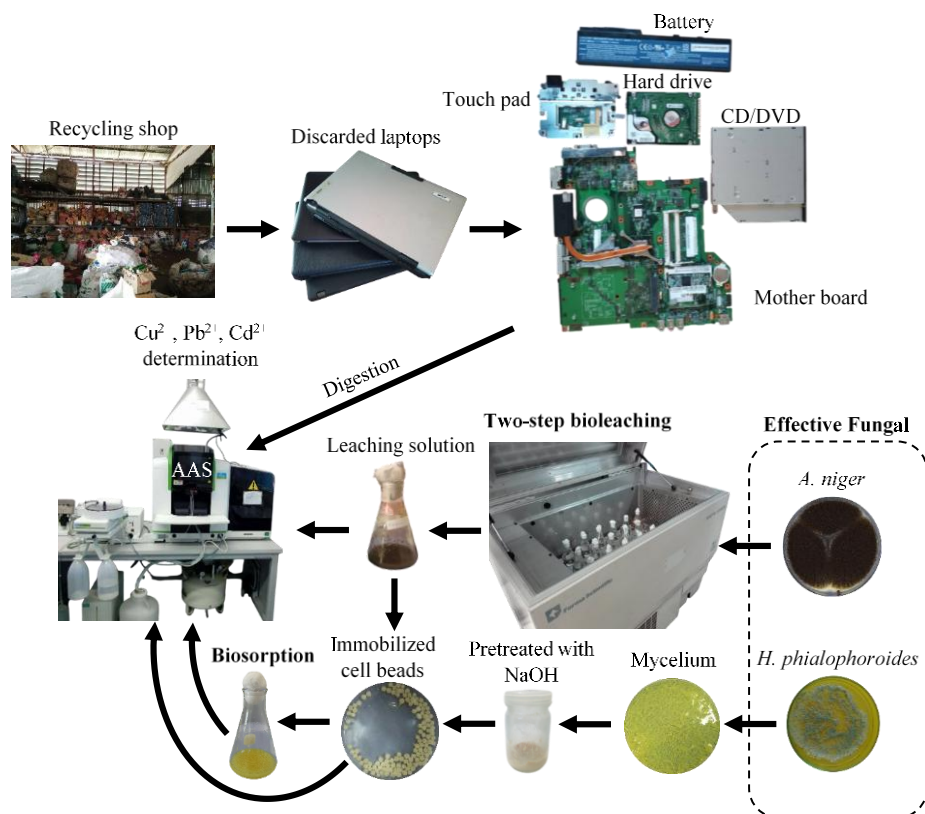


Fig. 1. illustrates the research methodology used in this study.

The general recommendation focuses on the digestion method, which was statistically derived based on the results found for all e-waste samples.

3.2. Variation of *A. niger* biomass, pH, citric acid, and heavy metal concentration with time during two-step bioleaching

During the seven days of *A. niger* growth, the pH of the medium decreased from 6 to 2.52 ± 0.02 , while the biomass dry weight rose to $511.68 \pm 3.22 \text{ mg L}^{-1}$, indicating that the fungus had entered its logarithmic growth phase and initiated the secretion of organic acids. After a 15-day incubation period, the medium's pH significantly decreased to 2.37 ± 0.09 ($p < 0.05$), while the biomass dry weight significantly increased to $552.59 \pm 8.16 \text{ mg L}^{-1}$ ($p < 0.05$). High-performance liquid chromatography (HPLC) analysis showed that the fungal culture achieved its peak citric acid production after 18 days at $97.27 \pm 0.73 \text{ mM L}^{-1}$ ($p < 0.05$). Comparison with pH measurements indicates that the fungus exhibited increased citric acid secretion at pH values below three. Conversely, when the medium's pH exceeded three, acid production declined (Table 1). Previous studies have reported that culture acidification is driven by citric acids produced by the *A. niger* strains, which play a crucial role in the metal bioleaching process [7,19]. These acids

contribute protons and metal-complexing anions, facilitating the release of free metal cations. When *A. niger* excretes citric acid during metal-recovery processes, the hydrogen ions from the citric acid initially lower the pH (acidolysis reactions). This is followed by the formation of metal complexes (complexolysis reactions). Once the citric acid is consumed, the pH begins to rise [20].

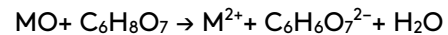
Acidolysis reactions:

- $\text{C}_6\text{H}_8\text{O}_7 \rightarrow \text{C}_6\text{H}_7\text{O}_7^- + \text{H}^+$ ($\text{pK}_{a1} = 3.09$)
- $\text{C}_6\text{H}_7\text{O}_7^- \rightarrow \text{C}_6\text{H}_6\text{O}_7^{2-} + \text{H}^+$ ($\text{pK}_{a2} = 4.75$)
- $\text{C}_6\text{H}_6\text{O}_7^{2-} \rightarrow \text{C}_6\text{H}_5\text{O}_7^{3-} + \text{H}^+$ ($\text{pK}_{a3} = 6.40$)

Complexolysis reactions:

- $n[\text{C}_6\text{H}_7\text{O}_7^-] + \text{M}^{n+} \rightarrow \text{M}[\text{C}_6\text{H}_7\text{O}_7]_n$
(Citric metallic complex)
- $n[\text{C}_6\text{H}_6\text{O}_7^{2-}] + 2\text{M}^{n+} \rightarrow \text{M}_2[\text{C}_6\text{H}_6\text{O}_7]_n$
(Citric metallic complex)
- $n[\text{C}_6\text{H}_5\text{O}_7^{3-}] + 3\text{M}^{n+} \rightarrow \text{M}_3[\text{C}_6\text{H}_5\text{O}_7]_n$
(Citric metallic complex)

The equation representing the reaction between metal (M) and citric acid is shown below, which leads to metal or M in the solution in an ionic form.



Citric acid was found to be more effective in recovering metals from Cu^{2+} in PCBs compared to Pb^{2+} and Cd^{2+} . At 21 days, the maximum leaching efficiencies were 73.06% for Cu^{2+} , 63.91% for Pb^{2+} , and 70.53% for Cd^{2+} ($p < 0.05$) (Table 2).

Table 1. Mycelium growth of *A. niger*, along with pH variation and citric acid production in nutrient broth, during two-step bioleaching of laptop PCBs over an incubation period of 27 days.

Time (day)	Dry mycelium weight (mg)	pH	Citric acid (mM L^{-1})
7*	511.68 ± 2.63^a	2.52 ± 0.02^{ab}	92.18 ± 1.51^{abc}
15	552.59 ± 8.16^{bc}	2.37 ± 0.09^a	95.93 ± 2.84^{bc}
18	556.30 ± 8.06^c	2.35 ± 0.14^{ab}	97.27 ± 0.73^c
21	553.30 ± 4.51^{bc}	2.57 ± 0.06^{ab}	91.23 ± 4.12^{ab}
24	541.86 ± 4.71^b	2.83 ± 0.07^b	91.16 ± 2.01^{ab}
27	541.62 ± 4.11^b	3.33 ± 0.33^c	89.12 ± 2.74^a

* Pre-cultivation of the cultures took 7 days before the addition of laptop PCBs. ** Means within a column with the same letter are not significantly different ($p < 0.05$).

Table 2. Heavy metals concentrations in RB broths during two-step bioleaching of laptop PCBs over an incubation period of 27 days.

Time (day)	Cu^{2+}		Pb^{2+}		Cd^{2+}	
	mg g^{-1} PCBs	%	mg g^{-1} PCBs	%	mg g^{-1} PCBs	%
15	204.12 ± 5.04^a	52.28	30.12 ± 0.54^a	52.01	3.22 ± 0.20^a	61.22
18	274.54 ± 8.2^b	70.31	32.65 ± 1.30^b	56.38	3.61 ± 0.04^b	68.63
21	285.26 ± 2.07^d	73.06	37.01 ± 0.29^d	63.91	3.71 ± 0.05^c	70.53
24	279.23 ± 3.10^c	71.51	36.11 ± 1.42^c	62.36	3.61 ± 0.03^b	68.63
27	278.21 ± 3.23^c	71.25	36.23 ± 1.40^c	62.56	3.60 ± 0.02^b	68.44

* Means within a column with the same letter are not significantly different ($p < 0.05$). ** The mean concentrations of Cu^{2+} , Pb^{2+} , and Cd^{2+} in 1 g of laptop PCBs were $390.45 \pm 7.02 \text{ mg}$, $57.91 \pm 5.22 \text{ mg}$, and $5.26 \pm 0.42 \text{ mg}$, respectively.

Citric acid has been shown to promote high leaching efficiencies, particularly in mobilizing Cu^{2+} , due to its ability to form complexes. Being a weak acid, it aids in their dissolution in water [21]. Additionally, citric acid has also yielded positive results in the leaching of Pb^{2+} and Cd^{2+} [22].

3.3. Optimum conditions of contact time, pH, and temperature for biosorption of heavy metals from leachate by *H. phialophoroides*

The leachate from the 21-day period by *A. niger*, which contained the highest metal concentrations, was selected for this part. The mean Cu^{2+} , Pb^{2+} , and Cd^{2+} concentrations in leachate with laptop PCBs were $285.26 \pm 2.07 \text{ mg g}^{-1}$ PCBs, $37.01 \pm 0.29 \text{ mg g}^{-1}$ PCBs, and $3.71 \pm 0.05 \text{ mg g}^{-1}$ PCBs, respectively. The results indicate that the *H. phialophoroides* biomass pretreated with NaOH exhibited lower metal ions removal efficiency from the leachate compared to the immobilized biomass combined with sodium alginate. The Cu^{2+} , Pb^{2+} , and Cd^{2+} adsorption by immobilized biomass over contact times that range from 30 to 180 minutes is demonstrated in Figure 2. Adsorption uptake improves with increasing contact time, reaching a maximum at 120 minutes ($p < 0.05$), after which it is constant. The maximum adsorption efficiencies

achieved were 43.06% for Cu^{2+} , 71.33% for Pb^{2+} , and 40.97% for Cd^{2+} using immobilized cells, whereas efficiencies of non-immobilized biomass were 39.52% for Cu^{2+} , 60.43% for Pb^{2+} , and 23.54% for Cd^{2+} . Several researchers have indicated that fungal biomass pretreatment and immobilization offer a straightforward technology for removing and recovering heavy metals from wastewater, with better suitability for reuse compared to non-immobilized biomass [23-26].

Immobilized cells are more effective than alginate for heavy metal removal by biomass due to an increase in the cell wall permeability. The many carboxy groups in alginates bind to the surface of fungal cells, increasing heavy metal ion adsorption capacity [11]. Additionally, immobilized cell beads can be handled more easily and reused in subsequent cycles [27]. The results revealed that the heavy metal biosorption by both adsorbents generally decreases as temperature rises, particularly at high temperatures above 50°C . At room temperature or 30°C , the maximum metal ion uptakes by the immobilized cells were $183.77 \pm 4.24 \text{ mg Cu}^{2+} \text{ g}^{-1}$ dry wt. and $2.28 \pm 0.03 \text{ mg Cd}^{2+} \text{ g}^{-1}$ dry wt., while Pb^{2+} was $45.27 \pm 1.83 \text{ mg Pb}^{2+} \text{ g}^{-1}$ dry wt. ($p < 0.05$) (Figure 3).

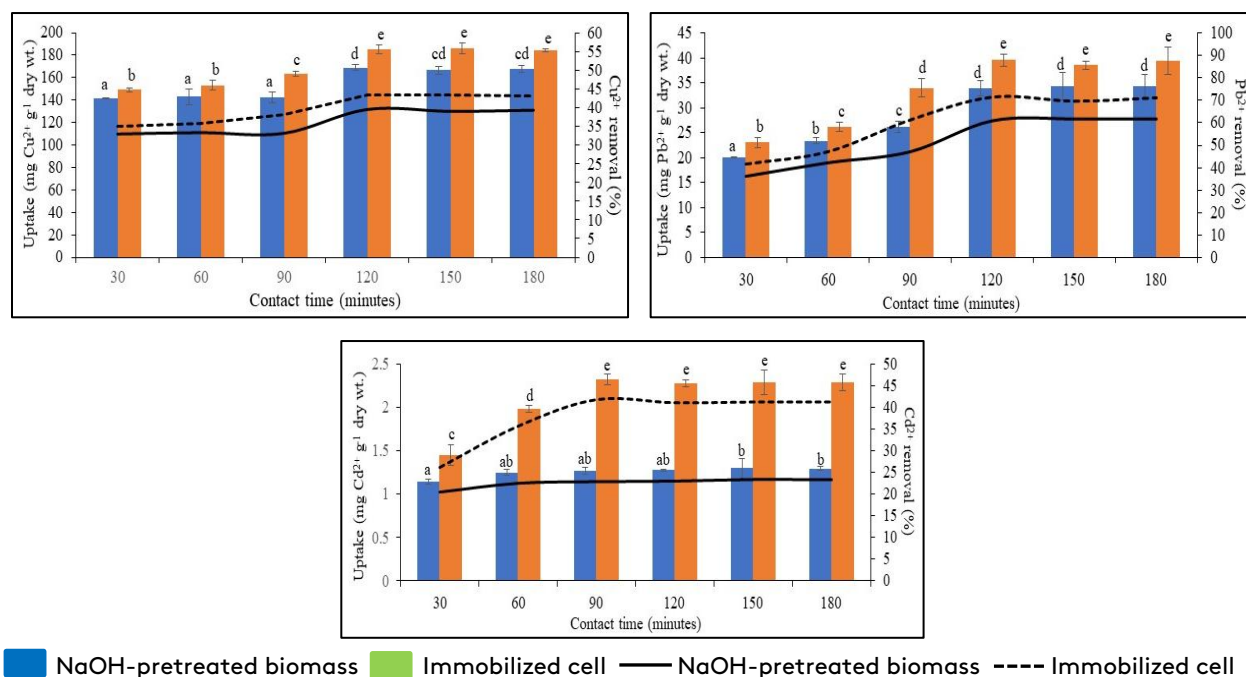


Fig. 2. Effect of contact time on the biosorption of mixed Cu^{2+} , Pb^{2+} , and Cd^{2+} ions from leachate by *H. phialophoroides*. The columns indicate heavy metal uptake (left y-axis), while the curves depict the percentage of heavy metal removal (right y-axis).

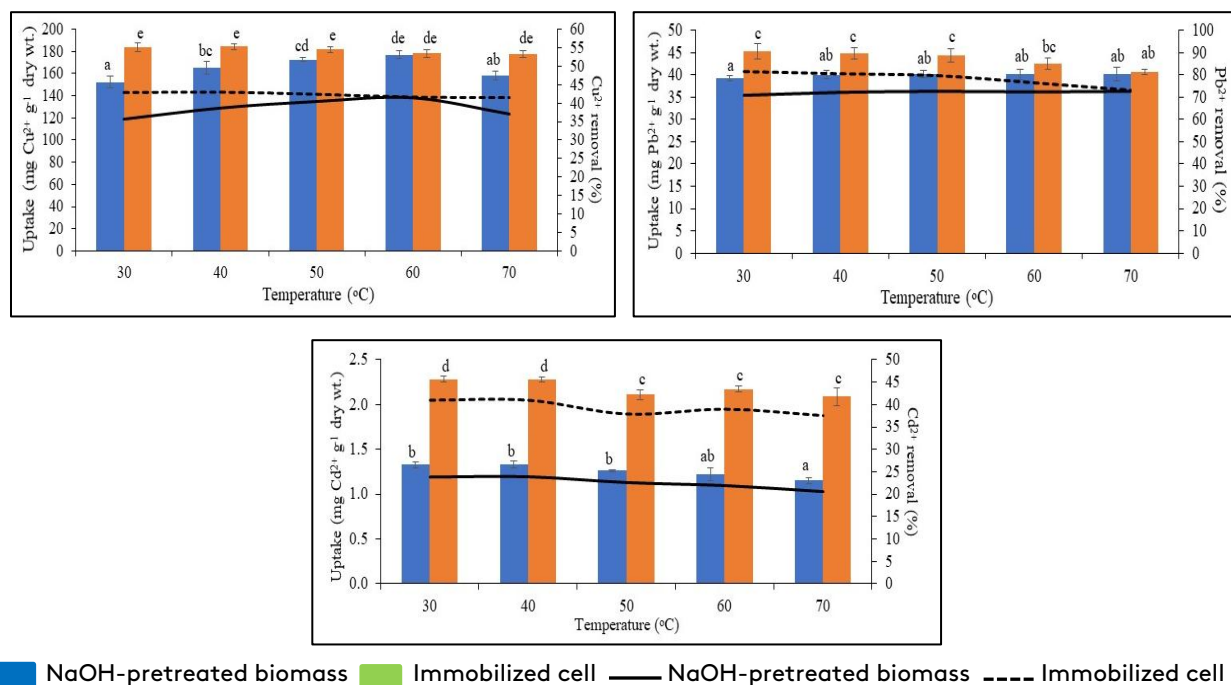


Fig. 3. Effect of temperature on the biosorption of mixed Cu^{2+} , Pb^{2+} , and Cd^{2+} ions from leachate by *H. phialophoroides*. The columns indicate heavy metal uptake (left y-axis), while the curves depict the percentage of heavy metal removal (right y-axis).

Elevated temperatures can cause distortion or damage to functional sites on cell surfaces and within the biomass. They can compromise cell membrane integrity and destabilize heavy metal-microorganism complexes, cell wall structures can be altered, and chemical groups on the cell wall can be ionized [28]. Similarly, Saad et al. [29] suggested that high temperatures significantly enhance Cu^{2+} , Pb^{2+} , Co^{2+} , Ni^{2+} , Fe^{3+} , and Cr^{3+} uptake by *Aspergillus tamarii* NRC 3.

Figure 4 illustrates that adsorption efficiencies from both adsorbents increased gradually from pH 3 to pH 7. The immobilized biomass achieved a higher metal ion uptake of $184.10 \pm 3.65 \text{ mg Cu}^{2+} \text{ g}^{-1} \text{ dry wt.}$, $39.60 \pm 1.21 \text{ mg Pb}^{2+} \text{ g}^{-1} \text{ dry wt.}$, and $2.28 \pm 0.04 \text{ mg Cd}^{2+} \text{ g}^{-1} \text{ dry wt.}$ at the optimal pH of 7 ($p < 0.05$).

Under acidic conditions, as the pH decreased, the adsorption capacity of both sorbents for heavy metals gradually declined. These findings are consistent with previous studies on various heavy metal removal using different immobilized fungal biomass [11,30]. The removal of ions from an aqueous solution is highly dependent on the solution's pH. The solution's pH value influences both the solubility and the ionization state of the

functional groups, such as amino, carboxylic, and phosphate groups, on the fungal cell wall. In addition, it influences the rate of ionization and the distribution of metal ions in the solution. The biosorption capacity typically increases with pH until it reaches the optimum level, where maximum biosorption occurs.

Beyond this point, further increases in pH lead to the precipitation of heavy metals because of the formation of heavy metal hydroxides or hydroxide anionic complexes [11,31].

The increase in the pH of the solution results in more protons detaching from the functional groups on the cell wall and enhancing the availability of negatively charged groups for complexation in response to increasing heavy metal ion adsorption.

The maximum heavy metal biosorption by the immobilized cells was achieved at a solution temperature of 30°C, pH 7, after 120 minutes of contact time. The biosorption capacities were $184.10 \pm 3.65 \text{ mg Cu g}^{-1} \text{ dry wt.}$, $39.60 \pm 1.21 \text{ mg Pb g}^{-1} \text{ dry wt.}$, and $2.28 \pm 0.04 \text{ mg Cd g}^{-1} \text{ dry wt.}$ Table 3 presents a general comparison of the results of this study with other research of adsorption of metal sorption capacities by different fungal species.

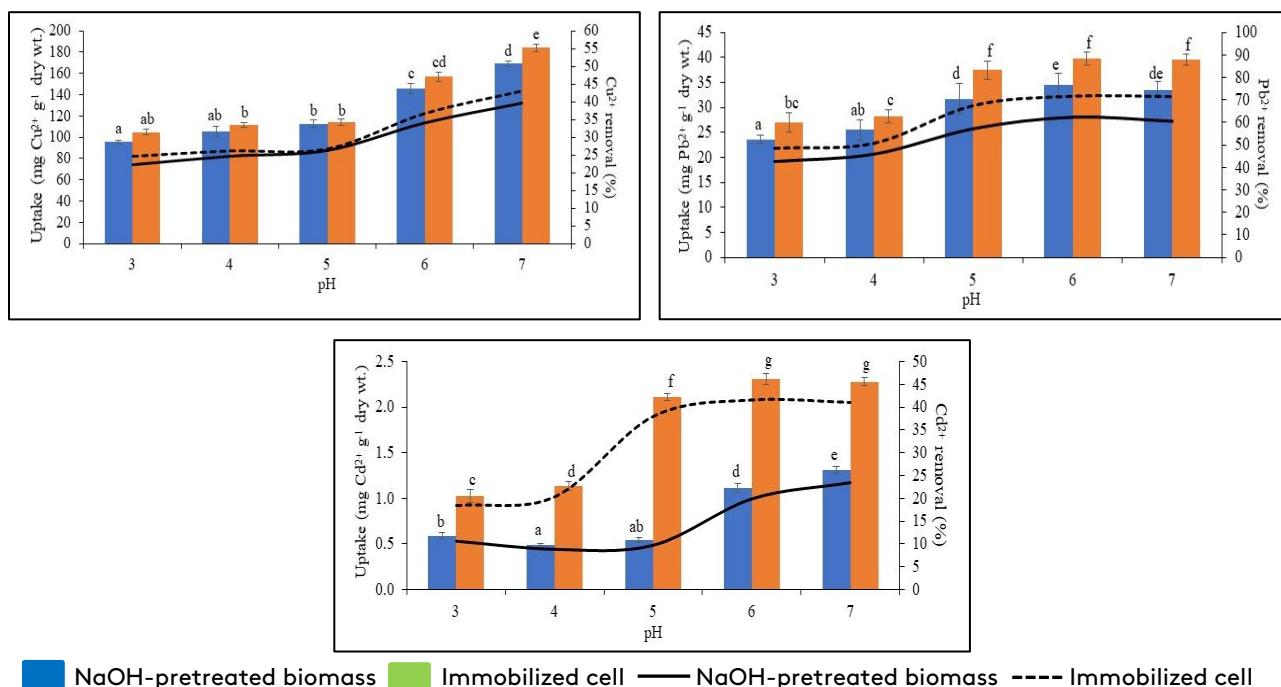


Fig. 4. Effect of pH on the biosorption of mixed Cu^{2+} , Pb^{2+} , and Cd^{2+} ions from leachate by *H. phialophoroides*. The columns indicate heavy metal uptake (left y-axis), while the curves depict the percentage of heavy metal removal (right y-axis).

Table 3. Maximum metal sorption capacities and operating conditions of immobilized fungal biomass.

Fungal biomass	Matrix	Metal ion	Concentration (mg L ⁻¹)	Max capacity (mg g ⁻¹)	pH	Temp. (°C)	Time (min)	Ref.
<i>Phanerochaete chrysosporium</i> (live cell)	Carboxymethylcellulose	Hg ²⁺	30-700	83.10	6	15-45	60	[32]
<i>Phanerochaete chrysosporium</i> (dead cell)	Carboxymethylcellulose	Hg ²⁺	30-700	102.15	6	15-45	60	[32]
<i>Cunninghamella echinulate</i> (NaOH-pretreated biomass)	Na alginate	Pb ²⁺	10-300	45	4	28	15	[30]
		Cu ²⁺	10-300	20	5	28	15	
		Zn ²⁺	10-300	18.8	5	28	15	
<i>Trichoderma asperellum</i>	Ca alginate	Cu ²⁺	50-600	140.85	5	28	480	[33]
<i>Penicillium sp.</i>	Chitosan	Cu ²⁺	25-2000	126.58	n.a	25	360	[34]
<i>Aspergillus niger</i>	Ca alginate	U ⁶⁺	10-200	694.4	5	30	540	[35]
<i>Rhizopus cohnii</i>	Na alginate /PVA, Fe ₃ O ₄	Cr ⁶⁺	5-200	6.97	1	37	480	[36]
<i>Humicola phialophoroides</i> (NaOH-pretreated biomass)	Na alginate	Cu ²⁺	285.26±2.07	184.10	7	30	120	This work
		Pb ²⁺	37.01±0.29	79.6				
		Cd ²⁺	3.71±0.05	2.28				

4. Conclusion

The two-step bioleaching experiment has shown that the fungus *A. niger* was capable of mobilizing heavy metals with high extraction efficiencies at pH below 3. Immobilized *H. phialophoroides* biomass pretreated with NaOH demonstrated higher decontamination efficiency than alginate in bioremediation processes. The maximum heavy metal biosorption by the immobilized cells was achieved at a solution temperature below 40 °C at pH 7 after an equilibrium time of 120 minutes.

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Author's contribution

Tinnapan Netpae was responsible for the conceptualization, methodology development, experimental investigation, data analysis, manuscript preparation, and final approval of the submitted version.

Conflict of interest

No potential conflict of interest was reported by the author.

Data availability

The datasets generated and/or analyzed during the current study are available with the corresponding author and shall be provided on reasonable request.

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