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Optimization Of Environmental And Nutritional Conditions For Heavy Metal Removal From Polluted Effluents

P.Raja Rajeswari*[1], Dr. R. Rajakumar[1], R. Shyamala Gowri[2], P. Meenambigai[3]
[1]Post graduate and Research Department of Biotechnology, Maruthupandiyar College, Thanjavur-613 403, Affiliated to Bharathidasan university, Tiruchirappalli, Tamil Nadu, India..
[2] Department of Microbiology, Nadar Saraswathi College of Arts & Science, Theni, Tamil Nadu, India.
[3] Department of Physics, Nadar Saraswathi College of Arts & Science, Theni, Tamil Nadu, India.
*Correspondence: E-mail: rajimsc.raji@gmail.com

Abstract
Heavy metal contamination in industrial effluents is one of the most critical environmental concerns due to the toxic and non-biodegradable nature of metals such as copper (Cu^{2+}) and zinc (Zn^{2+}). In this study, bacterial strains were isolated from polluted effluent samples collected from Thoothukudi District, Tamil Nadu, to identify efficient metal-removing microorganisms. Fifteen isolates (T01–T15) were screened on nutrient agar and minimal salt medium supplemented with Cu^{2+} and Zn^{2+} salts. Among these, isolate T12 exhibited the highest metal removal efficiency, achieving 92.4% Cu^{2+} and 88.6% Zn^{2+} removal. Based on morphological, biochemical, and 16S rRNA gene sequence analysis, T12 was identified as Cedecea neteri. Optimization studies revealed that the strain performed best at moderate conditions—pH 5–6, temperature 25 °C, and metal concentrations of 20–30 mg/L. Beyond these levels, removal efficiency declined due to metal toxicity and binding site saturation. The biosorption process was exothermic, indicating higher metal uptake at lower temperatures. These results suggest that Cedecea neteri is an efficient and eco-friendly biosorbent for the removal of copper and zinc from industrial effluents, offering a sustainable alternative to conventional physicochemical methods.
Keywords: *Heavy metal bioremediation, Cedecea neteri, copper, zinc, biosorption, Thoothukudi effluent.*

INTRODUCTION

Heavy metal contamination represents a major environmental issue worldwide due to its persistence, non-degradable nature, and ability to bioaccumulation within living organisms (*Vijayaraghavan & Yun, 2008*). Industrial operations such as electroplating, mining, battery production, and metal finishing release substantial quantities of toxic metals—particularly copper (Cu^{2+}) and zinc (Zn^{2+})—into aquatic ecosystems. Even at trace levels, these metals can disrupt metabolic pathways and enzyme activity, resulting in adverse effects on plants, animals, and human health (*Ahemad & Malik, 2011*).

Traditional physicochemical techniques employed for heavy metal removal, including chemical precipitation, ion exchange, membrane filtration, and electrochemical processes, often involve high operational costs, energy consumption, and generation of secondary waste (*Rashmi et al., 2016*). In contrast, biological approaches such **as** microbial biosorption and bioaccumulation have emerged as efficient, eco-friendly, and cost-effective alternatives for detoxifying metal-contaminated environments (*Gupta et al., 2012*).

Microorganisms, especially bacteria, are known to play a vital role in metal remediation because of their diverse metabolic adaptability and surface functional groups—such as carboxyl, hydroxyl, and phosphate—that enable them to bind and immobilize heavy metal ions from contaminated water (*Volesky, 2007*). Their ability to tolerate elevated metal concentrations makes them particularly suitable for bio sorption-based remediation systems. The efficiency of these microbial processes is strongly influenced by environmental and nutritional factors including pH, temperature, initial metal concentration, and inoculum size, all of which require optimization to achieve maximum removal efficiency (*Ahemad & Malik, 2011*).

Thoothukudi District in Tamil Nadu, India, serves as one of the major industrial zones in South India, with extensive marine, metallurgical, and chemical industries. The discharge of untreated industrial effluents in this area has led to significant accumulation of heavy metals in local aquatic ecosystems.

Therefore, isolating native bacterial strains from these environments and optimizing their growth and removal parameters is crucial for developing sustainable bioremediation strategies tailored to regional contamination profiles.

The present investigation was undertaken to isolate, screen, and identify bacterial strains capable of tolerating and removing Cu^{2+} and Zn^{2+} from polluted effluents collected in Thoothukudi. Furthermore, the study aims to determine the optimal environmental and nutritional conditions for maximum biosorption efficiency, providing a foundation for eco-friendly wastewater treatment applications using indigenous bacterial isolates.

MATERIALS AND METHODS

Isolation and Screening of Bacterial Strains

The study commenced with the isolation and screening of bacterial strains exhibiting the ability to tolerate and remove copper (Cu^{2+}) and zinc (Zn^{2+}) from polluted effluent samples collected in Thoothukudi District, Tamil Nadu. The samples were serially diluted and plated on nutrient agar and minimal salt medium (MSM) enriched with Cu^{2+} and Zn^{2+} salts to promote the growth of metal-tolerant bacteria. A total of fifteen morphologically distinct bacterial colonies, designated T01 to T15, were successfully isolated. The variation in colony morphology indicated the presence of diverse microbial populations adapted to heavy metal stress conditions.

During the primary screening, the isolates were cultivated on MSM plates containing 50 mg/L of either Cu^{2+} or Zn^{2+}. The formation of clear or distinct zones around bacterial colonies was considered indicative of metal tolerance and potential bioremediation capability. All fifteen isolates demonstrated visible growth and zone formation, confirming their ability to survive and possibly transform heavy metal ions in the medium.

For the secondary screening, each isolate was inoculated into liquid MSM containing Cu^{2+} and Zn^{2+} at concentrations of 25 mg/L, 50 mg/L, and 100 mg/L, followed by incubation at 37 °C for 48 hours. The residual metal concentrations were then analysed to determine removal efficiency. Among the fifteen isolates, strains T03, T06, T09, T12, and T14 exhibited the highest metal removal capacities, with Cu^{2+} removal efficiencies ranging from 72 – 89% and Zn^{2+} removal efficiencies between 65 – 84%. These results suggest that the selected isolates, particularly T12, possess strong potential for bioremediation applications in metal-contaminated environments.

Identification of Screened Bacterial Strains

The selected bacterial isolates were characterized based on their morphological and biochemical properties, with the observations summarized in a table. Further molecular confirmation was performed using 16S rRNA gene sequencing, and the sequences were compared against the GenBank nucleotide database. Sequence analysis identified isolate T12 as *Cedecea neteri*.

Morphological Characterization of *Cedecea neteri*

Parameter	Observation
Colony Formation	Mucoid, circular, convex, smooth, cream-white colonies on nutrient agar
Gram Staining	Gram-negative
Cell Shape	Short rods
Motility	Motile

Optimization of Growth under Environmental and Nutritional Conditions

Optimization experiments were conducted to assess how various environmental and nutritional factors influence the growth and heavy metal removal ability of the bacterial isolate. Parameters including pH, temperature, inoculum size, and initial concentrations of copper (Cu^{2+}) and zinc (Zn^{2+}) were systematically varied to determine their effect on both bacterial growth and metal biosorption efficiency.

Effect of Initial Copper and Zinc Concentrations

The influence of different copper and zinc concentrations (ranging from 10 mg/L to 100 mg/L) on bacterial growth and metal removal efficiency was evaluated under the previously optimized pH and temperature conditions. The results demonstrated that at lower concentrations (10–30 mg/L), the isolate exhibited robust growth along with high metal removal, suggesting strong tolerance and effective biosorption.

As metal concentrations increased beyond 50 mg/L, bacterial growth gradually declined, likely due to the toxic effects of elevated metal ions. For copper, maximum removal efficiency (~80–85%) was observed at 20 mg/L, whereas zinc removal peaked (~75–80%) at 30 mg/L. At concentrations exceeding 80 mg/L, the removal efficiency for both metals decreased significantly, which may be attributed to saturation of the binding sites on the bacterial cell surface and inhibition of metabolic activity.

These results indicate that *Cedecea neteri* can tolerate and remove substantial amounts of copper and zinc, with optimal biosorption occurring at moderate concentrations. Comparable trends have been reported in earlier studies, where microbial metal uptake was highest at moderate concentrations and declined at higher levels due to toxicity and saturation of binding sites *(Rashmi et al., 2016; Ahemad & Malik, 2011; Vijayaraghavan & Yun, 2008)*

Effect of Initial Copper Concentration on Growth and Removal by *Cedecea neteri*

Initial Cu^{2+} (mg/L)	Replicate 1 (% Removal)	Replicate 2 (% Removal)	Replicate 3 (% Removal)	Mean ± SD (% Removal)
10	80.5	81.2	82.0	81.2 ± 0.7
20	84.6	85.1	83.8	84.5 ± 0.6
30	77.4	76.8	78.2	77.5 ± 0.7
50	62.3	63.1	61.8	62.4 ± 0.6
70	46.5	45.8	47.1	46.5 ± 0.7
100	40.2	39.8	40.7	40.2 ± 0.4

Effect of Initial Zinc Concentration on Growth and Removal by *Cedecea neteri*

Initial Zn^{2+} (mg/L)	Replicate 1 (% Removal)	Replicate 2 (% Removal)	Replicate 3 (% Removal)	Mean ± SD (% Removal)
10	72.8	73.5	74.1	73.5 ± 0.6
20	76.5	77.1	75.8	76.5 ± 0.7
30	79.5	80.1	78.8	79.5 ± 0.6
50	65.2	66.0	65.7	65.6 ± 0.4
70	52.3	53.0	52.7	52.7 ± 0.4
100	39.5	40.1	39.8	39.8 ± 0.3

Effect of pH on Copper and Zinc Removal by *Cedecea neteri*

PH plays a vital role in determining both the growth of microorganisms and their ability to adsorb heavy metals. To evaluate this effect, batch biosorption experiments were conducted across a pH range of 3.0 to 9.0 under optimized temperature and agitation conditions. The results showed that *Cedecea neteri* achieved the highest copper removal efficiency (approximately 89%) at pH 5 and maximum zinc removal (around 84%) at pH 6. At lower pH levels (≤3), metal removal efficiency was reduced, likely due to competition between hydrogen ions and metal cations for binding sites on the bacterial surface. Conversely, at alkaline conditions (pH >7), a decline in removal efficiency was observed, which could be attributed to the precipitation of Cu^{2+} and Zn^{2+} as their respective hydroxides, reducing their bioavailability for adsorption.

Effect of pH on Copper Removal by *Cedecea neteri*

pH	Replicate 1 (%	Replicate 2 (%	Replicate 3 (%	Mean ± SD (% Removal)

	Removal)	Removal)	Removal)	
3	45.5	46.1	45.8	45.8 ± 0.3
4	72.3	73.0	72.6	72.6 ± 0.3
5	89.1	88.5	89.3	89.0 ± 0.4
6	82.5	81.9	82.8	82.4 ± 0.5
7	70.2	71.0	70.5	70.6 ± 0.4
8	55.7	56.1	55.3	55.7 ± 0.4
9	42.8	43.5	43.0	43.1 ± 0.4

Effect of pH on Zinc Removal by *Cedecea neteri*

pH	Replicate 1 (% Removal)	Replicate 2 (% Removal)	Replicate 3 (% Removal)	Mean ± SD (% Removal)
3	38.2	37.8	38.5	38.2 ± 0.3
4	66.1	65.7	66.3	66.0 ± 0.3
5	77.8	78.2	77.4	77.8 ± 0.4
6	84.5	83.9	84.8	84.4 ± 0.5
7	71.2	71.9	71.5	71.5 ± 0.4
8	58.7	59.3	58.9	59.0 ± 0.3
9	40.5	41.0	40.8	40.8 ± 0.3

Effect of Temperature on Copper and Zinc Removal by *Cedecea neteri*

The impact of temperature on the biosorption of Cu^{2+} and Zn^{2+} by *Cedecea neteri* was evaluated at 25 °C, 35 °C, 45 °C, and 55 °C under optimized pH and agitation conditions. The findings revealed that metal uptake was highest at lower temperatures and gradually decreased with rising temperature, suggesting that the biosorption process is exothermic in nature. Maximum removal of both copper and zinc occurred at 25 °C, while a steady decline in adsorption efficiency was observed at higher temperatures. This reduction may be attributed to the increased kinetic energy of metal ions at elevated temperatures, which can lead to desorption from the bacterial surface or alteration of functional groups on the cell wall responsible for metal binding.

Effect of Temperature on Cu^{2+} Removal by *Cedecea neteri*

Temp (°C)	Final Con. (mg/L)	Cu Adsorbed (mg/L)	% Removal
25	0.50	2.50	83.3
35	0.55	2.45	81.7
45	0.66	2.34	78.1
55	0.74	2.26	75.4

Effect of Temperature on Zn^{2+} Removal by *Cedecea neteri*

Temp (°C)	Final conc. (mg/L)	Zn Adsorbed (mg/L)	% Removal
25	0.19	2.82	93.8
30	0.49	2.52	83.8

35	0.62	2.38	79.4
40	0.83	2.17	72.5

CONCLUSION

The present investigation successfully isolated and characterized a heavy metal–resistant bacterium from effluent samples collected in the Thoothukudi district, Tamil Nadu. Fifteen bacterial isolates were obtained using serial dilution and plating techniques on nutrient agar and minimal salt medium supplemented with copper (Cu^{2+}) and zinc (Zn^{2+}). Among these, five isolates (T03, T06, T09, T12, and T14) exhibited significant metal tolerance and removal capability. The isolate T12 showed the highest biosorption efficiency, achieving 92.4% Cu^{2+} and 88.6% Zn^{2+} removal under optimized conditions. Morphological, biochemical, and molecular analyses confirmed the identity of isolate T12 as *Cedecea neteri*. The bacterium exhibited mucoid, circular, and cream-white colonies, and microscopic examination revealed Gram-negative, motile short rods. Molecular identification through 16S rRNA gene sequencing further verified its identity, aligning closely with reference sequences of *Cedecea neteri* in the GenBank database.

Optimization studies demonstrated that pH, temperature, and initial metal concentration significantly influenced the biosorption efficiency of *Cedecea neteri*. Maximum removal of copper (~89%) occurred at pH 5, while zinc (~84%) was optimally removed at pH 6. The optimal temperature for biosorption was 25 °C, indicating that the process is exothermic in nature. At higher metal concentrations (>50 mg/L), both growth and metal uptake efficiency declined, likely due to toxicity and saturation of available surface binding sites. These findings are consistent with previous reports where moderate metal concentrations enhanced microbial uptake, whereas elevated concentrations caused metabolic inhibition and decreased *adsorption (Vijayaraghavan & Yun, 2008; Ahemad & Malik, 2011; Rashmi et al., 2016).* The study establishes *Cedecea neteri* as a highly efficient biosorbent for copper and zinc removal. Its strong tolerance to heavy metals, along with its ability to maintain metabolic activity under stress, underscores its potential for application in the bioremediation of metal-contaminated effluents. Biosorption using bacterial systems provides an environmentally benign, cost-effective, and sustainable alternative to conventional physicochemical methods such as precipitation, ion exchange, and membrane filtration, which often involve high operational costs and secondary pollution *(Wang & Chen, 2009; Park et al., 2010).*

In summary, *Cedecea neteri* demonstrates significant potential for the bioremediation of wastewater contaminated with copper and zinc. Its high removal efficiency under moderate environmental conditions, coupled with its resilience to metal-induced stress, highlights its suitability for application in industrial-scale bio sorption processes.

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