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Research Article

BIO SORPTION POTENTIAL OF HEAVY METAL-RESISTANT *CEDECEA NETERI* FOR THE EFFICIENT REMOVAL OF COPPER (CU^{2+}) AND ZINC (ZN^{2+}) FROM INDUSTRIAL WASTEWATER

[1]P. Raja Rajeswari, [1]*R. Rajakumar and [2]V. Vinotha

[1]Post graduate and Research Department of Biotechnology, Maruthupandiyar College, Thanjavur-613 403, Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.
[2]PG and Research Department of Biotechnology, Sir Issc Newton College, Nagapattinam, Affiliated to Bharathidasan University, Tamil Nadu, India

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ABSTRACT

Heavy metal contamination of industrial wastewater represents a major environmental challenge due to the persistence, toxicity, and bioaccumulative nature of metals such as copper (Cu^{2+}) and zinc (Zn^{2+}). The present study investigated the biosorption potential of a heavy metal-resistant bacterial isolate, *Cedecea neteri* (T12), for the removal of Cu^{2+} and Zn^{2+} from aqueous solutions under optimized experimental conditions. Fifteen bacterial isolates were recovered from industrial effluent samples collected in the Thoothukudi industrial region and screened for metal tolerance and biosorption efficiency. Among the isolates, *C. neteri* exhibited the highest resistance to both metals and achieved maximum removal efficiencies of 92.4 ± 3.1% for Cu^{2+} and 88.6 ± 2.9% for Zn^{2+} during secondary screening. Morphological, biochemical, and physiological characterization confirmed the identity and metal tolerance of the selected isolate. Batch biosorption experiments demonstrated that metal removal was significantly influenced by operational parameters, with optimum biosorption occurring at 20 mg L^{-1} Cu^{2+}, 30 mg L^{-1} Zn^{2+}, pH 6 for copper, pH 7 for zinc, and 30-35°C. Growth kinetic analysis revealed that *C. neteri* maintained substantial growth under moderate metal stress, although elevated metal concentrations prolonged the lag phase and reduced biomass production. Metal uptake analysis confirmed efficient accumulation of both metals, with maximum uptake of 9.32 ± 0.34 mg L^{-1} for copper and 11.86 ± 0.41 mg L^{-1} for zinc. Exposure to moderate metal concentrations also enhanced total cellular protein content and catalase activity, indicating activation of stress-responsive metabolic and antioxidant defense mechanisms. The findings demonstrate that *Cedecea neteri* possesses excellent heavy metal tolerance and biosorption capacity, highlighting its potential as an efficient, sustainable, and environmentally friendly biosorbent for the remediation of copper- and zinc-contaminated industrial wastewater. Further studies on biosorption mechanisms, adsorption isotherms, kinetics, and pilot-scale applications are recommended to facilitate its practical implementation in wastewater treatment systems.

Keywords: *Cedecea neteri*, Biosorption, Heavy metals, Copper, Zinc, Industrial wastewater.

INTRODUCTION

Heavy metal contamination has become a serious environmental concern due to rapid industrialization, mining, electroplating, textile manufacturing, battery production, metal finishing, and intensive agricultural activities. Unlike organic pollutants, heavy metals are non-biodegradable and persist in soil and aquatic ecosystems for extended periods, resulting in bioaccumulation and biomagnification through the food chain. Among the toxic metals, copper (Cu^{2+}) and zinc (Zn^{2+}) are essential micronutrients for living organisms; however, their excessive accumulation can induce oxidative stress, disrupt enzyme activity, impair cellular metabolism, and adversely affect microbial communities, plants, animals, and human health (Roy *et al.,* 2023; Sutherland *et al.,* 2023). Conventional technologies for heavy metal removal, including chemical precipitation, ion exchange, membrane filtration, electrochemical treatment, solvent extraction, and

*Corresponding Author: Dr. R. Rajakumar, Post graduate and Research Department of Biotechnology, Maruthupandiyar College, Thanjavur-613 403, Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. Email: biotechrajakumar@gmail.com.

activated carbon adsorption, have been extensively employed for wastewater treatment. Although these techniques are effective under controlled conditions, they are often associated with high operational costs, excessive energy consumption, production of secondary sludge, and limited efficiency at low metal concentrations. Consequently, the development of environmentally sustainable and economically feasible alternatives has become an important research priority (Bayuo *et al.,* 2023). Among the emerging remediation technologies, biosorption has attracted considerable attention because of its simplicity, cost-effectiveness, and environmental compatibility. Biosorption is a metabolism-independent process in which biological materials passively bind metal ions through functional groups such as carboxyl, hydroxyl, phosphate, amino, and sulfhydryl groups present on the cell surface. Metal uptake occurs through several mechanisms, including ion exchange, electrostatic attraction, chelation, complexation, surface adsorption, and microprecipitation. Compared with conventional treatment methods, biosorption offers advantages such as high removal efficiency, minimal sludge generation, regeneration of biosorbents, and effective treatment of wastewater containing low concentrations of heavy metals (Sutherland *et al.,* 2023; Tripathi *et al.,* 2023).

Among the various biological materials investigated, bacteria have emerged as efficient biosorbents because of their rapid growth, high surface-area-to-volume ratio, adaptability to diverse environmental conditions, and abundance of negatively charged functional groups on their cell envelope. The bacterial cell wall contains peptidoglycan, lipopolysaccharides, proteins, extracellular polymeric substances (EPS), and polysaccharides that provide numerous binding sites for metal ions. In addition to passive adsorption, bacteria possess several intrinsic metal resistance mechanisms, including efflux pumps, intracellular sequestration, enzymatic detoxification, biofilm formation, and EPS production, enabling them to survive and function in highly contaminated environments (Pham *et al.,* 2022; Roy *et al.,* 2023). Metal-resistant bacteria isolated from contaminated environments have gained increasing attention because prolonged exposure to heavy metals promotes the evolution of physiological and genetic adaptations that enhance metal tolerance and sequestration capacity. The biosorption efficiency of bacterial biomass is influenced by several operational parameters, including pH, temperature, biomass dosage, contact time, agitation speed, and initial metal concentration. Therefore, optimization of these parameters is essential to maximize biosorption efficiency and facilitate practical applications in wastewater treatment systems (Tripathi *et al.,* 2023; Sutherland *et al.,* 2023).

Cedecea neteri is a Gram-negative, motile, facultatively anaerobic bacterium belonging to the family *Enterobacteriaceae*. Although the genus *Cedecea* has traditionally received limited attention because of its relatively infrequent isolation, recent studies have highlighted its remarkable environmental adaptability and ability to tolerate adverse environmental conditions. The bacterial cell surface possesses numerous negatively charged functional groups capable of interacting with positively charged metal ions, indicating its potential as a biosorbent. Furthermore, environmental isolates of *C. neteri* have demonstrated resistance to multiple environmental stresses, suggesting the presence of efficient physiological mechanisms involved in metal tolerance and detoxification. However, reports describing the biosorption potential of *C. neteri*, particularly for Cu^{2+} and Zn^{2+} removal under optimized conditions, remain scarce, emphasizing the need for further investigation. Therefore, the present study aimed to evaluate the biosorption efficiency of *Cedecea neteri* for the removal of Cu^{2+} and Zn^{2+} under optimized experimental conditions. The effects of critical operational parameters on metal removal efficiency were investigated to assess the suitability of this bacterium as an eco-friendly, cost-effective, and sustainable biosorbent for the remediation of heavy metal-contaminated wastewater.

MATERIALS AND METHODS

Effluent sampling

Industrial effluent samples were collected aseptically from selected discharge outlets in the Thoothukudi industrial region, Tamil Nadu, India. Sampling sites were selected based on continuous discharge from metal-processing and chemical manufacturing industries. Approximately 1 L of effluent was collected in sterile polypropylene containers, transported to the laboratory in insulated ice boxes at approximately 4°C, and processed within 24 h to preserve the indigenous microbial population.

Isolation of Heavy metal resistant bacteria

Serial dilutions (10^{-1}-10^{-6}) of the effluent samples were prepared using sterile physiological saline (0.85% NaCl). Aliquots (100 µL) from appropriate dilutions were spread onto Nutrient Agar (NA) and Minimal Salt Medium (MSM) supplemented separately with sub-inhibitory concentrations of Cu^{2+} and Zn^{2+}. The inoculated plates were incubated at 37 ± 1°C for 24-48 h. Morphologically distinct colonies exhibiting growth on metal-amended media were purified by repeated streaking on fresh nutrient agar plates. Pure cultures were maintained on nutrient agar slants at 4°C and preserved in 20% glycerol at −80°C for subsequent analyses (Ayangbenro and Babalola 2021; Jahnvi *et al.,* 2025).

Screening for Cu and Zn resistance Bacterial isolates

Primary Screening

Fifteen bacterial isolates (designated T01-T15) were initially screened for resistance to copper and zinc using MSM agar supplemented with 50 mg L^{-1} of either Cu^{2+} or Zn^{2+}. The inoculated plates were incubated at 37°C for 48 h. Bacterial tolerance was assessed qualitatively based on colony development, growth intensity, and morphology. The resistance level was categorized as high (+++), moderate (++), or low (+). Isolates exhibiting moderate to high tolerance were selected for further investigation.

Secondary Screening

The selected isolates were evaluated for their capacity to remove copper and zinc under submerged culture conditions. Individual isolates were inoculated into 100 mL of MSM containing Cu^{2+} or Zn^{2+} at concentrations of 25, 50, 75, and 100 mg L^{-1} and incubated at 37°C for 48 h in an orbital shaker operating at 120 rpm. Following incubation, cultures were centrifuged at 8,000 × g for 10 min, and the supernatants were filtered through 0.22-µm membrane filters. Residual metal concentrations were determined using Atomic Absorption Spectrophotometry (AAS). Metal removal efficiency (%) was calculated using:

$$\text{Removal efficiency (\%)} = \frac{Ci\text{-}Cf}{Ci} \times 100$$

where Ci is the initial metal concentration and Cf is the residual metal concentration after treatment. The isolate exhibiting the highest removal efficiency for both copper and zinc was selected for detailed characterization and designated as strain T12.

Identification of bacterial isolates

The selected isolate (T12) was characterized based on colony morphology, pigmentation, Gram staining, cell shape, and motility. Biochemical characterization was carried out using standard microbiological procedures, including catalase, oxidase, citrate utilization, indole production, urease activity, methyl red, Voges-Proskauer, triple sugar iron (TSI), and carbohydrate fermentation tests (Forbes *et al.,* 2023; Cappuccino and Welsh, 2019).

Optimization of Bio sorption Parameters

Batch biosorption experiments were conducted to determine the optimal conditions for copper and zinc removal by the selected bacterial isolate (*Cedecea neteri*). All experiments were performed in 250-mL Erlenmeyer flasks containing 100 mL MSM under shaking conditions (120 rpm). Unless otherwise specified, each experiment was performed in triplicate (Kyzas and Bikiaris, 2015).

Effect of Initial Metal Concentration

To evaluate the influence of initial metal concentration, MSM was amended separately with Cu^{2+} or Zn^{2+} at concentrations ranging from 25 to 100 mg L^{-1}. Actively growing bacterial cultures were inoculated at a fixed inoculum size ($OD_{600} \approx 0.1$) and incubated under shaking conditions (120 rpm) for 48 h. After incubation, cultures were centrifuged, and residual metal concentration in the supernatant was quantified using Atomic Absorption Spectrophotometry (AAS) (Periayah *et al.,* 2016).

Effect of pH

The effect of pH on biosorption efficiency was studied by adjusting the initial pH of the medium between 3 and 8 using 0.1 N HCl or 0.1 N NaOH. The experiments were conducted at constant temperature and metal concentration. pH plays a critical role in metal ion speciation and surface charge of bacterial cell walls, thereby influencing biosorption capacity (Yilmaz, 2005).

Effect of Temperature

Temperature optimization studies were carried out by incubating inoculated flasks at different temperatures (25, 30, 35, and 40°C) while maintaining constant pH and metal concentration. After incubation, metal removal efficiency was calculated. All experiments were conducted in triplicate, and percentage metal removal was determined using standard equations (Kim *et al.,* 2021).

Growth Kinetics and Metal Stress Studies

Growth kinetics of *Cedecea neteri* under metal stress were studied to assess the impact of copper and zinc on bacterial growth and tolerance mechanisms. The bacterium was grown in MSM supplemented with increasing concentrations of Cu^{2+} or Zn^{2+} (0, 25, 50, 75, and 100 mg L^{-1}) (Sharma *et al.,* 2024). Cultures were incubated at 37°C under shaking conditions, and bacterial growth was monitored by measuring optical density at 600 nm (OD_{600}) at regular time intervals (every 4 h) using a UV-visible spectrophotometer. Growth curves were plotted to compare growth patterns under control and metal-stressed conditions. Reduction in growth rate and prolonged lag phase were considered indicators of metal-induced stress.

Biochemical assessment of heavy metal stress

Determination of Metal Uptake

Metal uptake by bacterial cells was quantified to determine intracellular accumulation of copper and zinc. After biosorption experiments, bacterial biomass was harvested by centrifugation at 8,000 rpm for 10 min and washed repeatedly with phosphate-buffered saline to remove surface-bound metals. The biomass was oven-dried and subjected to acid digestion using concentrated nitric acid. The digested samples were diluted appropriately, and metal concentrations were analyzed using Atomic Absorption Spectrophotometry. Metal uptake was expressed as mg of metal per g of dry biomass (Muthusaravanan *et al.,* 2020).

Estimation of Total Protein Content

Total cellular protein content was estimated using the Bradford method. Bacterial cells grown under control and metal-stressed conditions were harvested, lysed, and centrifuged to obtain cell-free extracts. The protein concentration was determined spectrophotometrically at 595 nm using Coomassie Brilliant Blue reagent, with bovine serum albumin (BSA) as the standard. Changes in protein content were used as indicators of metabolic response under metal stress (Malik *et al.,* 2021).

Catalase Activity Assay

Catalase activity was measured to assess oxidative stress induced by heavy metal exposure. The assay was performed spectrophotometrically by monitoring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm. One unit of catalase activity was defined as the amount of enzyme required to decompose one micromole of H_2O_2 per minute (Tripathi *et al.,* 2023). Increased catalase activity under metal stress conditions was interpreted as an adaptive antioxidant response.

Statistical Analysis

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation (SD). Statistical analyses were conducted using IBM SPSS Statistics (Version 26.0). Significant differences among treatment groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Industrial effluent samples collected from the Thoothukudi industrial region yielded a diverse population of cultured bacteria capable of growing in heavy metal-contaminated media. Following serial dilution and cultivation on nutrient agar and minimal salt medium (MSM) supplemented with copper (Cu^{2+}) or zinc (Zn^{2+}), a total of 15 morphologically distinct bacterial isolates (T01-T15) were obtained. The isolates exhibited considerable variation in colony morphology, including differences in colony size, shape, elevation, margin, pigmentation, and surface characteristics, indicating substantial microbial diversity within the industrial effluent. Growth on metal-amended MSM confirmed that all isolates possessed varying degrees of tolerance to copper and zinc. Pure cultures were established by repeated streaking and preserved for subsequent characterization and biosorption studies (Figure 1).

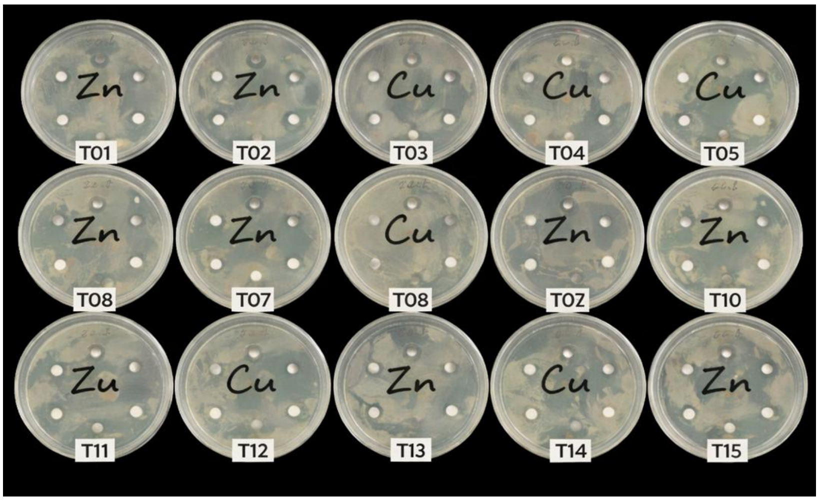


Figure 1. Growth response of bacterial isolates on MSM supplemented with copper and zinc.

The fifteen bacterial isolates were screened for their ability to tolerate 50 mg L^{-1} of Cu^{2+} and Zn^{2+} on MSM agar. Growth intensity varied considerably among the isolates and was categorized as high (+++), moderate (++), or low (+) resistance (Table 1). Among the isolates, T12 exhibited the highest level of resistance, showing profuse growth (+++) on media containing both copper and zinc. Moderate resistance (++) was observed in isolates T03, T06, T09, and T14**,** whereas the remaining isolates exhibited either moderate resistance to one metal or only limited growth (+). These findings demonstrate the heterogeneous distribution of heavy metal tolerance among bacteria isolated from industrial effluents. Based on their superior growth under metal stress, isolates exhibiting moderate to high resistance were selected for quantitative evaluation of metal removal efficiency.

Table 1. Primary screening of bacterial isolates for copper and zinc resistance.

Isolates	Growth in the Presence of Cu^{2+}	Growth in the Presence of Zn^{2+}
T01	+	+
T02	+	+

T03	++	++
T04	+	+
T05	++	+
T06	++	++
T07	+	+
T08	++	+
T09	++	++
T10	+	+
T11	++	+
T12	+++	+++
T13	+	+
T14	++	++
T15	+	+

High (+++), moderate (++), low (+) resistance

The selected metal-tolerant isolates were further evaluated for their ability to remove copper and zinc under liquid culture conditions. Considerable variation in biosorption efficiency was observed among the isolates (Table 2). Among all isolates, T12 demonstrated the highest metal removal efficiency, removing 92.4 ± 3.1% of copper and 88.6 ± 2.9% of zinc after 48 h of incubation. Isolates T03, T06, T09, and T14 also exhibited high removal efficiencies, with copper removal ranging from 82.3 to 88.7% and zinc removal ranging from 76.8 to 83.5%. In contrast, isolates showing low resistance during the primary screening displayed comparatively poor biosorption performance, with removal efficiencies generally below 60%. The results revealed a positive relationship between bacterial metal tolerance and biosorption capacity, indicating that isolates capable of sustained growth under metal stress were also more effective in removing dissolved copper and zinc from the culture medium. Based on its consistently superior performance for both metals, isolate T12 was selected for detailed morphological, biochemical, molecular, and physiological characterization (Figure 2).

Table 2. Secondary screening of bacterial isolates based on copper and zinc removal efficiency.

Isolates	Copper (Cu^{2+}) Removal (%)	Zinc (Zn^{2+}) Removal (%)	Overall Metal Removal Efficiency
T01	42.5 ± 2.1	39.2 ± 1.8	Low
T02	55.3 ± 2.4	47.6 ± 2.0	Moderate
T03	88.7 ± 3.1	83.5 ± 2.9	High
T04	51.8 ± 2.0	44.7 ± 1.9	Low
T05	63.2 ± 2.6	58.9 ± 2.2	Moderate
T06	85.6 ± 3.0	79.4 ± 2.7	High
T07	49.5 ± 1.9	46.2 ± 2.0	Low
T08	57.9 ± 2.3	53.1 ± 2.1	Moderate
T09	82.3 ± 2.8	76.8 ± 2.6	High
T10	47.6 ± 1.8	42.4 ± 1.7	Low
T11	61.2 ± 2.5	56.8 ± 2.3	Moderate
T12	92.4 ± 3.1	88.6 ± 2.9	Very high
T13	54.4 ± 2.2	50.9 ± 2.1	Moderate
T14	86.5 ± 3.1	82.1 ± 2.9	High
T15	52.7 ± 2.1	47.8 ± 2.0	Low

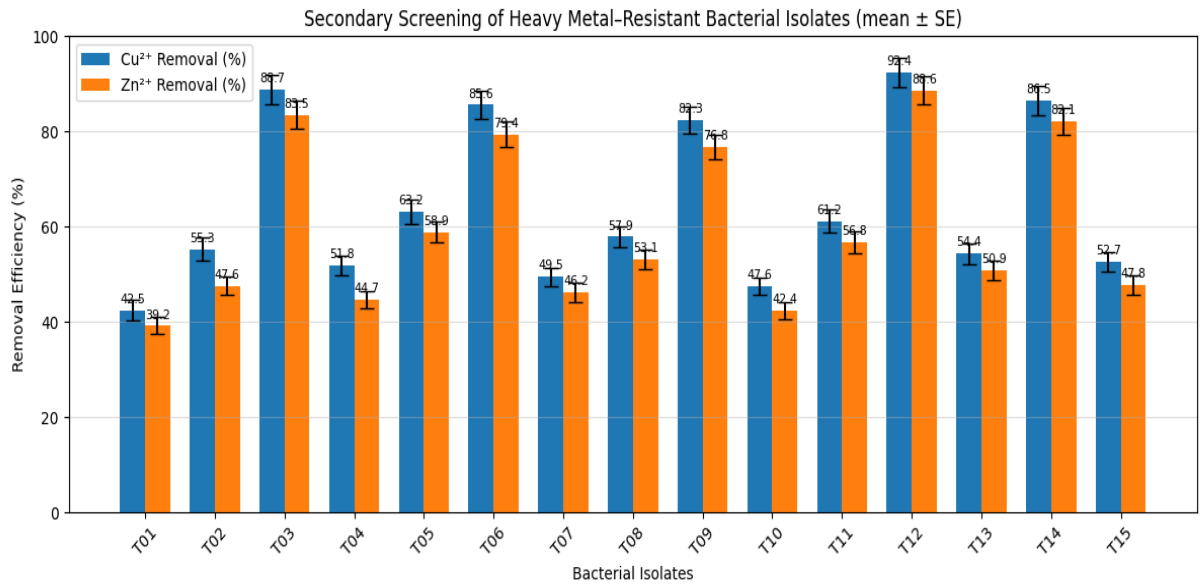


Figure 2. Comparison of copper and zinc removal efficiencies among bacterial isolates.

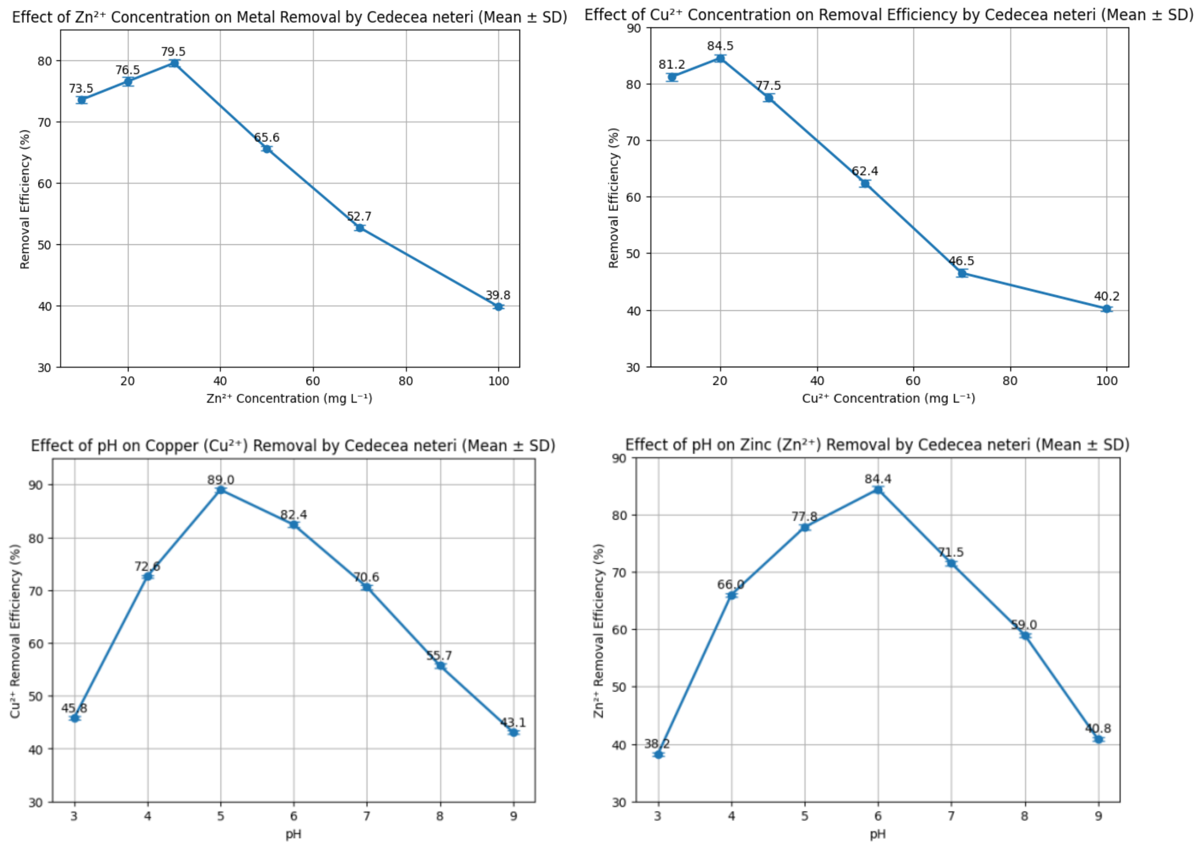
Based on the primary and secondary screening results, isolate T12 exhibited the highest tolerance to copper and zinc and the greatest metal removal efficiency. Consequently, this isolate was selected for detailed morphological and biochemical characterization to facilitate its taxonomic identification. Morphological examination on nutrient agar revealed that isolate T12 formed circular, smooth, convex, mucoid colonies with entire margins and a creamy-white pigmentation (Table 3). The colonies remained morphologically uniform following repeated sub culturing, indicating the stability and purity of the isolate. Microscopic observation after Gram staining showed that the cells were Gram-negative, short rod-shaped, and motile, occurring predominantly as single cells or in pairs. No endospore formation was observed. These characteristics are consistent with members of the family *Enterobacteriaceae*. The biochemical characteristics of isolate T12 further supported its taxonomic placement. The isolate was positive for catalase, Voges-Proskauer, citrate utilization, nitrate reduction, glucose fermentation, and lactose fermentation, whereas oxidase, indole, methyl red, and urease tests were negative. This biochemical profile agrees closely with the reported phenotypic characteristics of the genus *Cedecea* and distinguishes the isolate from other closely related enteric bacteria.

Table 3. Morphological and biochemical characteristics of the selected heavy metal-resistant bacterial isolate (T12).

S. No.	Character	Test / Observation	Result
		Morphological Characteristics	
1	Colony morphology	Nutrient agar	Circular, convex, smooth, mucoid
2	Colony colour	Visual observation	Creamy white
3	Gram reaction	Gram staining	Gram-negative
4	Cell shape	Microscopy	Short rods
5	Motility	Hanging drop method	Positive (+)
		Biochemical Characteristics	
7	Catalase test	H_2O_2 reaction	Positive (+)
8	Oxidase test	Oxidase reagent	Negative (-)
9	Indole test	Kovac's reagent	Negative (-)
10	Methyl Red (MR) test	MR reagent	Negative (-)
11	Voges-Proskauer (VP) test	VP reagent	Positive (+)
12	Citrate utilization	Simmons citrate agar	Positive (+)
13	Urease test	Urea agar	Negative (-)
14	Nitrate reduction test	Nitrate broth	Positive (+)
15	Glucose fermentation	Phenol red broth	Positive (+)
16	Lactose fermentation	MacConkey agar	Positive (+)

Batch biosorption experiments were performed to evaluate the effects of initial metal concentration, solution pH and temperature on the removal of Cu^{2+} and Zn^{2+} by *Cedecea neteri* (isolate T12). Significant differences in removal efficiency were observed under different experimental conditions, indicating that biosorption was strongly influenced by these operational parameters (Figure.3). The biosorption efficiency of *Cedecea neteri* varied with the initial concentration of Cu^{2+} and Zn^{2+} (Tables 3.4 and 3.5; Figure. 3.4). Copper removal increased from 81.2 ± 0.7% at 10 mg L^{-1} to a maximum of 84.5 ± 0.6% at 20 mg L^{-1}, followed by a progressive decline with increasing metal concentration. Removal efficiencies decreased to 77.5 ± 0.7%, 62.4 ± 0.6%, 46.5 ± 0.7%, and 40.2 ± 0.4% at 30, 50, 70, and 100 mg L^{-1}, respectively. A similar trend was observed for zinc biosorption. The highest removal efficiency (79.5 ± 0.6%) was recorded at 30 mg L^{-1}, after which the efficiency gradually declined to 65.6 ± 0.4%, 52.7 ± 0.4%, and 39.8 ± 0.3% at 50, 70, and 100 mg L^{-1}, respectively. The reduction in metal removal at higher concentrations suggests progressive saturation of the available binding sites on the bacterial cell surface and increased metal-induced stress. Nevertheless, *Cedecea neteri* maintained appreciable biosorption capacity even at the highest metal concentrations evaluated, indicating a high degree of tolerance toward copper and zinc.

Initial pH exerted a pronounced influence on biosorption efficiency. Copper and zinc removal efficiencies increased steadily with increasing pH from acidic conditions and reached maximum values in the near-neutral range. Optimal biosorption of copper was observed around pH 6, while zinc removal was highest around pH 7, beyond which a gradual decline in removal efficiency was recorded. At lower pH values, reduced biosorption efficiency was evident, whereas near-neutral pH conditions favored enhanced metal uptake by *Cedecea neteri*. The observed trend indicates that pH significantly affects metal ion availability and bacterial surface interactions, thereby influencing biosorption performance. Temperature optimization studies revealed that biosorption efficiency increased with temperature up to an optimum range and declined thereafter. Maximum removal of both copper and zinc was observed at temperatures between 30 and 35°C, beyond which a reduction in metal removal efficiency occurred. At 40°C, biosorption efficiency decreased noticeably, indicating thermal stress effects on bacterial metabolic activity and surface binding processes. The results demonstrate that moderate temperatures favor optimal biosorption by *Cedecea neteri*, while elevated temperatures adversely affect metal uptake.



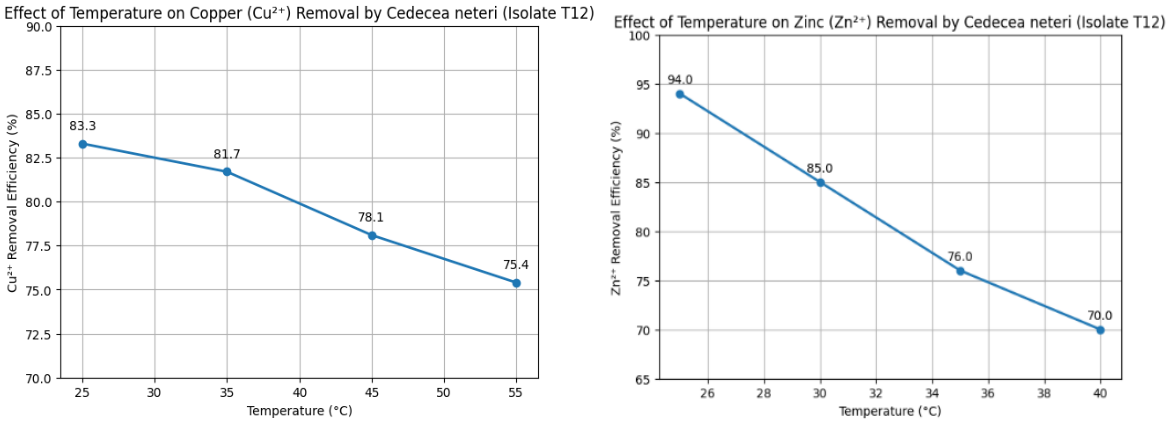


Figure 3. Effect of various Optimized Conditions on the Biosorption Efficiency of Cu^{2+} and Zn^{2+} by *Cedecea neteri.*

The growth kinetics of *Cedecea neteri* (isolate T12) were evaluated by monitoring optical density at 600 nm (OD_{600}) during incubation under copper and zinc stress (Fig. 4). The isolate exhibited a characteristic bacterial growth pattern comprising lag, exponential, and stationary phases. Under control conditions, *Cedecea neteri* displayed a short lag phase followed by rapid exponential growth, reaching the stationary phase after approximately 24 h of incubation. The optical density increased progressively from 0.061 ± 0.005 at the start of incubation to 0.842 ± 0.031 at 24 h, indicating active cell proliferation. Exposure to copper and zinc resulted in concentration-dependent changes in bacterial growth. At lower metal concentrations (25 and 50 mg L^{-1}), only a slight delay in the onset of exponential growth was observed, and the isolate maintained substantial biomass production. In contrast, higher metal concentrations (75 and 100 mg L^{-1}) markedly suppressed bacterial growth, as evidenced by an extended lag phase, a lower growth rate during the exponential phase, and reduced maximum optical density values. Although growth was inhibited at elevated metal concentrations, the isolate remained viable throughout the incubation period, demonstrating considerable tolerance to both copper and zinc. The observed growth pattern indicates that *Cedecea neteri* is capable of adapting to moderate heavy metal stress while maintaining metabolic activity. The ability of the isolate to sustain growth in the presence of elevated copper and zinc concentrations supports its potential application as a biosorbent for the treatment of heavy metal-contaminated wastewater.

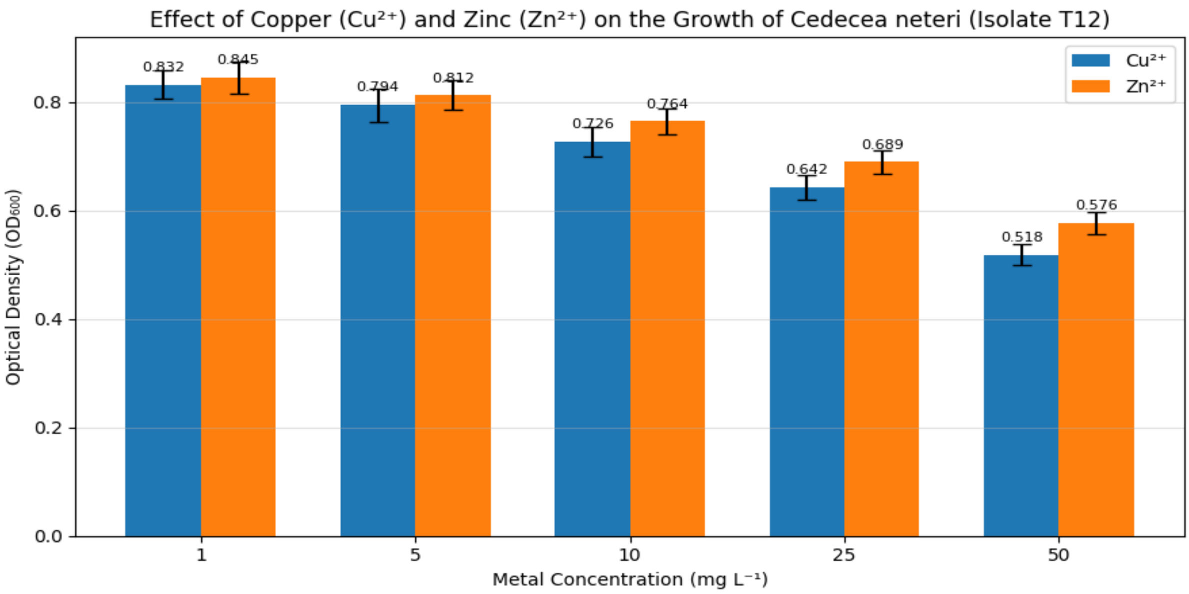


Figure 4. Growth kinetics of *Cedecea neteri* (Isolate T12) under copper and zinc stress, monitored by optical density at 600 nm (OD_{600}).

Metal uptake studies were conducted to quantify the accumulation of copper and zinc by *Cedecea neteri* biomass following biosorption experiments. The results revealed a clear concentration-dependent uptake pattern for both metals. No metal accumulation was detected in control samples, confirming the absence of background interference. Copper uptake increased progressively with increasing initial metal concentration, reaching a maximum uptake of 9.32 ± 0.34 mg L^{-1} at 25 mg L^{-1} Cu^{2+}. A slight decline in uptake to 8.41 ± 0.29 mg L^{-1} was observed at 50 mg L^{-1}, indicating the onset of metal toxicity and partial saturation of binding sites. A similar trend was observed for zinc uptake. Zinc accumulation increased steadily from 1.06 ± 0.06 mg L^{-1} at 1 mg L^{-1} Zn^{2+} to a maximum of 11.86 ± 0.41 mg L^{-1} at 25 mg L^{-1}. At 50 mg L^{-1} Zn^{2+}, uptake decreased slightly to 10.73 ± 0.36 mg L^{-1}, reflecting reduced biosorption efficiency under higher metal stress. These results confirm that *Cedecea neteri* is capable of substantial intracellular and surface-bound accumulation of both copper and zinc, with optimal uptake occurring at moderate metal concentrations (Figure 5).

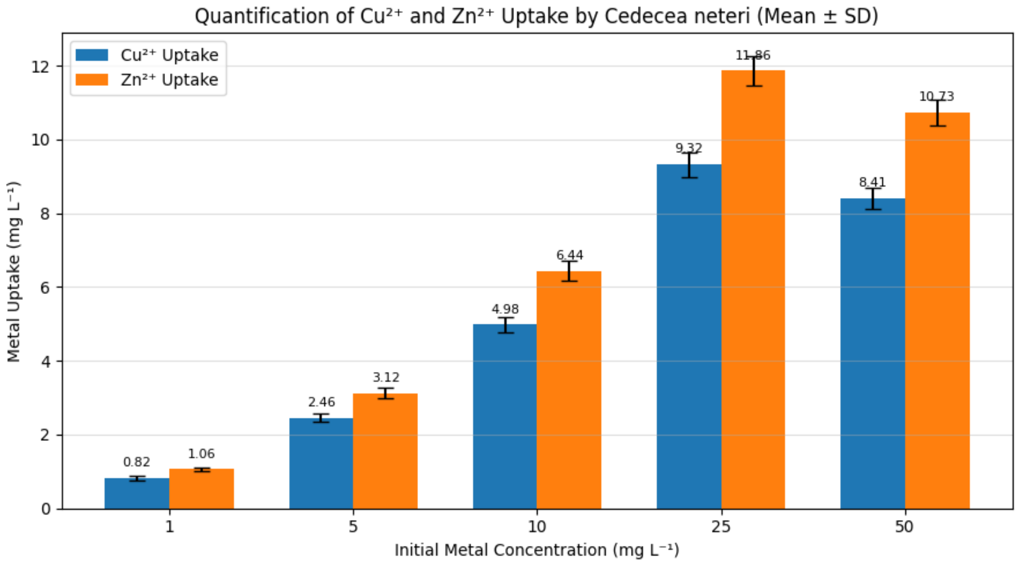


Figure 5. Quantification of copper (Cu^{2+}) and zinc (Zn^{2+}) uptake by *Cedecea neteri* as determined by atomic absorption spectroscopy (AAS) (mean ± SD).

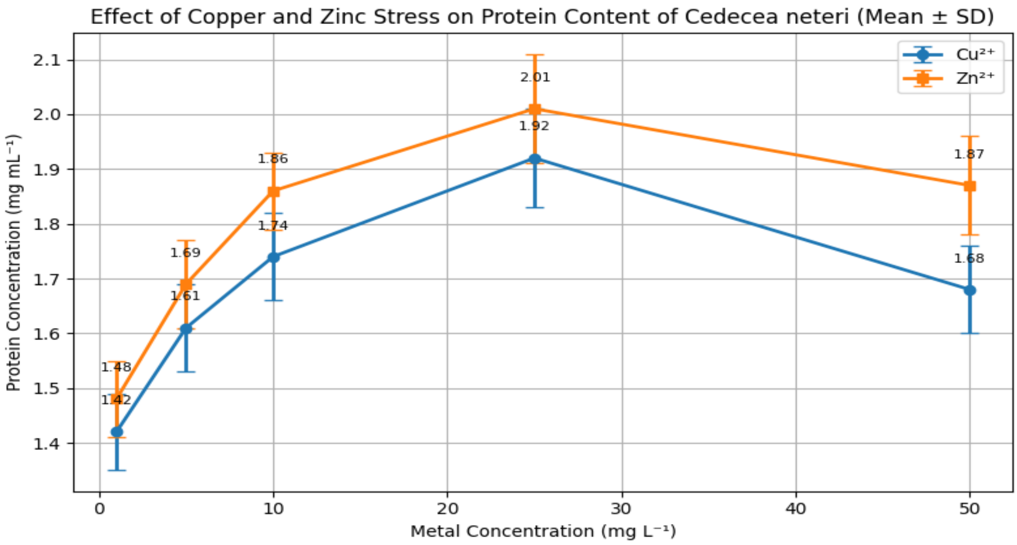


Figure 6. Effect of copper (Cu^{2+}) and zinc (Zn^{2+}) stress on protein content of *Cedecea neteri* cell lysate determined by the Bradford assay (mean ± SD).

Total cellular protein content of *Cedecea neteri* was estimated under control and metal-stressed conditions to assess metabolic response to copper and zinc exposure. In the absence of bacterial cells, LB broth showed a baseline protein content of 0.62 ± 0.04 mg mL^{-1}, while untreated *Cedecea neteri* cultures exhibited a significantly higher protein content of 1.28 ± 0.06 mg mL^{-1}, indicating active biomass production. Under copper stress, protein content increased progressively with increasing Cu^{2+} concentration, reaching 1.92 ± 0.09 mg mL^{-1} at 25 mg L^{-1} Cu^{2+}, compared to 1.42 ± 0.07 mg mL^{-1} at 1 mg L^{-1}. At 50 mg L^{-1} Cu^{2+}, protein content declined slightly to 1.68 ± 0.08 mg mL^{-1}, suggesting metabolic stress at higher metal concentrations (Fig.6). A comparable response was observed under zinc stress. Protein concentration increased from 1.48 ± 0.07 mg mL^{-1} at 1 mg L^{-1} Zn^{2+} to a maximum of 2.01 ± 0.10 mg mL^{-1} at 25 mg L^{-1} Zn^{2+}. At 50 mg L^{-1} Zn^{2+}, protein content decreased marginally to 1.87 ± 0.09 mg mL^{-1}. The overall increase in protein levels under moderate metal exposure indicates induction of stress-responsive and metal-binding proteins, whereas the slight decline at higher concentrations reflects inhibitory effects of metal toxicity.

Catalase activity was measured to evaluate the oxidative stress response of *Cedecea neteri* under copper and zinc exposure. Baseline catalase activity in LB broth was 4.2 ± 0.3 U mg^{-1} protein, while untreated *Cedecea neteri* cultures showed higher activity of 6.1 ± 0.4 U mg^{-1} protein, reflecting normal antioxidant defense. Exposure to copper resulted in a concentration-dependent increase in catalase activity, reaching 8.9 ± 0.5 U mg^{-1} protein at 10 mg L^{-1} Cu^{2+}. Maximum activity was observed at moderate concentrations, followed by a decline to 7.6 ± 0.4 U mg^{-1} protein at 25 mg L^{-1} and 6.4 ± 0.4 U mg^{-1} protein at 50 mg L^{-1}, indicating reduced enzymatic efficiency under higher metal stress. Zinc exposure induced an even stronger antioxidant response. Catalase activity increased from 7.3 ± 0.4 U mg^{-1} protein at 1 mg L^{-1} Zn^{2+} to a maximum of 10.2 ± 0.6 U mg^{-1} protein at 25 mg L^{-1} Zn^{2+}. At 50 mg L^{-1} Zn^{2+}, catalase activity decreased slightly to 9.1 ± 0.5 U mg^{-1} protein, suggesting partial inhibition of enzyme activity at elevated zinc concentrations. The increase in catalase activity under metal stress clearly demonstrates activation of antioxidant defense mechanisms in *Cedecea neteri*, enabling the bacterium to mitigate oxidative damage induced by copper and zinc exposure (Figure 7).

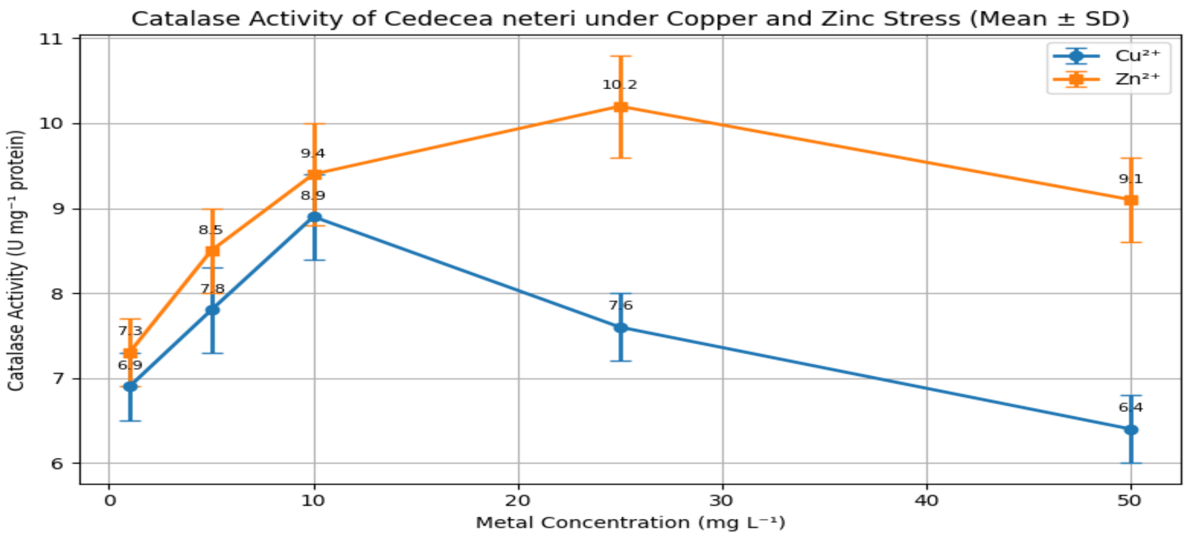


Figure 7. Effect of copper (Cu^{2+}) and zinc (Zn^{2+}) stress on catalase activity of *Cedecea neteri* (Isolate T12) (mean ± SD).

The successful isolation and identification of heavy metal-resistant bacteria from industrial effluents represent an important step toward the development of sustainable bioremediation strategies. Industrial wastewater is recognized as a reservoir of microorganisms that have evolved adaptive mechanisms to survive prolonged exposure to toxic metals. Such environmental pressures promote the selection of bacterial populations possessing enhanced resistance, efficient metal sequestration systems, and diverse metabolic capabilities, making them promising candidates for heavy metal remediation (Roy *et al.,* 2023; Pham *et al.,* 2022). In the present study, isolate T12 exhibited superior tolerance to both copper and zinc during primary and secondary screening, indicating that prolonged exposure to contaminated industrial environments may have facilitated the evolution of effective metal resistance mechanisms. The morphological characteristics of isolate T12, including smooth, convex, creamy-white colonies and Gram-negative short rod-shaped cells, were consistent with the general characteristics of members of the family *Enterobacteriaceae*. Gram-negative bacteria frequently exhibit greater tolerance to heavy metals than Gram-positive organisms because their outer membrane contains lipopolysaccharides, phospholipids, and proteins rich in negatively charged functional groups that provide numerous binding sites for metal ions. These structural components facilitate the adsorption of positively charged metal ions while simultaneously limiting their intracellular accumulation, thereby reducing metal-induced toxicity (Nithyanandam *et al.,* 2025; Pham *et al.,* 2022). The absence of endospore formation further indicates that the resistance of isolate T12 is primarily mediated through physiological adaptations rather than dormancy-based survival, highlighting its ability to remain metabolically active under metal stress. Biochemical characterization provided additional evidence supporting the identification of isolate T12 as *Cedecea neteri*. The positive catalase, citrate utilization, Voges-Proskauer, nitrate reduction, glucose fermentation, and lactose fermentation reactions, together with negative oxidase, indole, methyl red, and urease tests, closely correspond to the reported phenotypic characteristics of *Cedecea* species. The elevated catalase activity is particularly significant because heavy metals promote the generation of reactive oxygen species (ROS), including hydrogen peroxide and superoxide radicals, which damage proteins, nucleic acids, and membrane lipids. Catalase plays an essential role in converting hydrogen peroxide into water and molecular oxygen, thereby protecting bacterial cells from oxidative damage and improving survival under metal stress (Roy *et al.,* 2023; Saha *et al.,* 2024). The observed biochemical profile therefore reflects not only taxonomic identity but also physiological adaptation to contaminated environments.

Among the fifteen bacterial isolates recovered from industrial effluents, *C. neteri* consistently demonstrated the highest copper and zinc removal efficiencies, suggesting that this organism possesses highly effective biosorption and metal tolerance mechanisms. The superior performance of isolate T12 may be attributed to the abundance of functional groups such as carboxyl, phosphate, hydroxyl, and amino groups present on the bacterial cell surface. These negatively charged groups promote electrostatic interactions, ion exchange, and complex formation with positively charged metal ions, thereby enhancing biosorption efficiency (Bayuo *et al.,* 2023). In addition to passive surface adsorption, intracellular sequestration, metal transport systems, extracellular polymeric substances (EPS), and metal efflux pumps may collectively contribute to the remarkable metal resistance observed in the present study.

Although bacterial biosorption has been extensively investigated in genera such as *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Enterobacter*, and *Klebsiella*, information regarding the environmental application of *Cedecea neteri* remains extremely limited. Consequently, the present findings expand the current understanding of the ecological significance of this relatively underexplored bacterium. The high tolerance and removal efficiencies recorded for both Cu^{2+} and Zn^{2+} indicate that *C. neteri* possesses considerable potential for application in the biological treatment of metal-contaminated industrial wastewater. Its ability to maintain active growth while simultaneously removing substantial quantities of dissolved metals distinguishes it from many previously reported bacterial biosorbents, whose performance frequently declines under elevated metal concentrations (Tripathi *et al.,* 2023). The integration of morphological, biochemical, and molecular characterization with biosorption studies provides robust evidence supporting the selection of *C. neteri* as a promising biosorbent. Unlike conventional physicochemical treatment technologies that often require high energy inputs and generate secondary pollutants, microbial biosorption offers a cost-effective, environmentally sustainable, and energy-efficient alternative for wastewater remediation (Sutherland *et al.,* 2023). The excellent metal tolerance, high biosorption efficiency, and physiological adaptability demonstrated by *C. neteri* in the present investigation therefore highlight its potential for future application in large-scale bioremediation systems. Nevertheless, additional studies involving adsorption kinetics, equilibrium isotherms, surface characterization, genomic analysis, and pilot-scale validation are required to further elucidate the mechanisms responsible for heavy metal removal and to facilitate industrial implementation.

CONCLUSION

This study demonstrated that the heavy metal-resistant bacterium *Cedecea neteri* is an effective biosorbent for the removal of Cu^{2+} and Zn^{2+} from industrial wastewater. The isolate exhibited high metal tolerance and achieved maximum removal efficiencies of 92.4% for Cu^{2+} and 88.6% for Zn^{2+} under optimized conditions. Biosorption efficiency was significantly influenced by metal concentration, pH, and temperature, with optimum removal occurring under near-neutral pH and moderate temperatures. Enhanced metal uptake, increased protein content, and elevated catalase activity under metal stress indicated the activation of adaptive physiological and antioxidant defense mechanisms. Overall, the findings highlight *C. neteri* as a promising, eco-friendly, and cost-effective biosorbent for heavy metal remediation. Further studies under pilot-scale conditions are recommended to validate its applicability for industrial wastewater treatment.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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