

Research Article

A Protocol for Elucidating the Mechanisms of Biosorption: Uptake of Cu(II) and Pb(II) by Viable *Pseudomonas aeruginosa* Cells

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Article Info	Abstract
Article History	This study presents a methodological approach to elucidate the mechanisms underlying the biosorption of Cu(II) and Pb(II) by live <i>Pseudomonas aeruginosa</i> cells. Experiments were conducted under controlled conditions, and kinetic, equilibrium, and desorption analyses were employed to characterise both transport and attachment mechanisms. The biosorption of Pb(II) was best represented by the pseudo-second-order model, while the fit of Cu(II) was best described by both the pseudo-first- and pseudo-second-order models. The diffusion-chemisorption (D-C) model provided additional insight into transport mechanisms. The solid-phase mass transfer index (R_{DC}) values indicated that intraparticle diffusion played a moderate role in controlling the uptake of both Cu(II) and Pb(II), with a more pronounced effect observed for Pb(II). Equilibrium data conformed best to the Sips isotherm, suggesting heterogeneous surface binding and possible cooperative adsorption. Maximum monolayer capacities were 82.48 mg/g for Cu(II) and 32.50 mg/g for Pb(II). Desorption studies enabled the quantitative attribution of metal binding to distinct interactions, including physisorption, ion exchange, complexation, and intracellular accumulation. Cu(II) biosorption was primarily governed by complexation (51%) and ion exchange (46%), while Pb(II) biosorption was largely driven by ion exchange (64%). The approach formulated in this research offers a practical framework for probing biosorption mechanisms in subsequent investigations.
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1. Introduction

Our natural water resources continue to decline in quality due to persistent pollutants, including heavy metals and organic compounds, from industrial, agricultural, and domestic sources [1]. This deterioration in the quality of the natural environment poses a risk to human and ecological health [2]. Interception of waste streams followed by treatment is one possible way to eliminate the contamination; however, this solution is often a financial burden to industries, particularly small and medium-sized industries unable to sustain exorbitant treatment costs [3]. As a result, untreated effluents are often discharged into the environment, leading to widespread heavy metal pollution.

Numerous studies have documented alarming concentrations of heavy metals in the environment that exceed established safety limits. For instance, Withanachchi et al. [4] reported mean levels of copper, nickel, zinc, and lead in surface waters of the Mashavera River Basin, Georgia, reaching 13,158, 180.3, 12,505, and 69.6 µg/L, respectively. In another study, lead levels in groundwater and soil from the Thane Region of Maharashtra, India, were reported at concentrations of 8.9 µg/L and 49.2 mg/kg, respectively [5]. The concentration of heavy metals in industrial effluents can vary significantly and is often dependent on the industry. For instance, an analysis of tannery sludge collected from Gazipur, Bangladesh, revealed mean concentrations of chromium, lead, and copper reaching 20,900, 62.2, and 420.4 mg/kg, respectively [6].

The prolonged exposure to heavy metals in the environment or workplace can also lead to serious health consequences. Forsyth et al. [7] identified lead contamination in pregnant women at levels exceeding 5 µg/L due to contaminated food storage cans. In another study, it was shown that there was a potential carcinogenic risk to both adults and children from cadmium and arsenic exposure from wastewater treatment plant sludge [8]. The impact on human health from heavy metal exposure depends on both the dose and the specific heavy metal. Lead exposure is known to impair the nervous system, reduce memory function, and contribute to cardiovascular disease [9]. Hexavalent chromium has been reported to cause cardiovascular, neurological, and neuronal toxicity [10]. In excess, copper may trigger neurodegenerative disorders [11]. While this summary highlights key toxicological effects of heavy metal exposure, more detailed discussions are available in studies by Rehman et al. [9] and Mitra et al. [12].

Adsorption technologies have shown strong potential for the removal of point- and non-point-source heavy metal contaminations. Within this area, biosorption using microbial biomass has emerged as an eco-compatible and cost-effective solution. However, understanding the mechanisms governing biosorption is critical for advancing microbial-based technologies for heavy metal remediation. Insights into the transport and attachment mechanisms can aid process optimisation, improve predictive modelling, and guide system design. In this work, a protocol was developed to elucidate the mechanisms involved in the removal of heavy metal ions from solution. *Pseudomonas aeruginosa*, a model bacterium, was employed in controlled batch experiments in which Cu(II) and Pb(II) were selected as model cations. The biosorption transport mechanisms were examined through a combination of experimental procedures and mathematical modelling, while attachment mechanisms were evinced via chemical desorption using various eluents.

Additionally, kinetic and equilibrium studies were conducted to optimise the biosorption process. This protocol is intended as an additional resource for researchers seeking to deepen their understanding of biosorption mechanisms.

2. Literature Review

Increased global awareness of the environmental and health risks associated with heavy metals has fuelled extensive research into more efficient and economically viable treatment technologies [13]. Traditional methods such as chemical precipitation, electrocoagulation, reverse osmosis, and ion exchange have shown considerable potential for industrial use [14]. Many of these approaches suffer notable limitations, including high energy demands, secondary sludge generation, challenges in achieving low residual concentrations, and elevated operational costs [15]. Among alternative techniques, adsorption is often highlighted as a cost-effective option. Medfu Tarekegn et al. [16], in their review of the use of microbes for bioremediation of heavy metals, iterated that bacteria are a crucial candidate for heavy metal remediation. Further, the authors emphasised that to advance this technology, more studies are needed to elucidate the exact mechanisms involved in heavy metal remediation. Both Gram-positive and Gram-negative bacteria have demonstrated effective capabilities in heavy metal removal. For example, Gram-negative *Enterobacter ludwigii* was used by Black et al. [17] to achieve removal efficiencies of 84% for Cu(II) and 98% for Pb(II). In a separate study, Ray et al. [18] reported 96% Pb(II) removal from solution using the Gram-positive *Bacillus cereus*.

The structural characteristics of bacterial cell walls play a significant role in their absorptive performance. The cell wall, which is located outside the cytoplasmic membrane, is composed primarily of peptidoglycan, a mesh-like polymer of cross-linked polysaccharides and peptide chains [19]. This basic structure is shared among both Gram-negative and Gram-positive bacteria; however, Gram-negative species possess an additional outer lipid membrane enveloping the peptidoglycan layer (Figure 1). This outer membrane contains phospholipids and lipopolysaccharides, which contribute to its unique permeability and structural complexity [20]. *Pseudomonas aeruginosa* is a Gram-negative, rod-shaped bacterium belonging to the Pseudomonadaceae family and thrives in diverse environments, including soil, water, and hospital settings [21, 22]. Bacterial sorption of heavy metals generally occurs through two main mechanisms. First, passive biosorption, also known as extracellular binding, often involves the reversible,

rapid attachment of metal ions to cell walls and does not require metabolic activity [23]. The second is active biosorption, a metabolism-dependent process that includes bioaccumulation and biotransformation, where metal ions penetrate the cell membrane. Unlike passive uptake, this intracellular binding is typically slower [24].

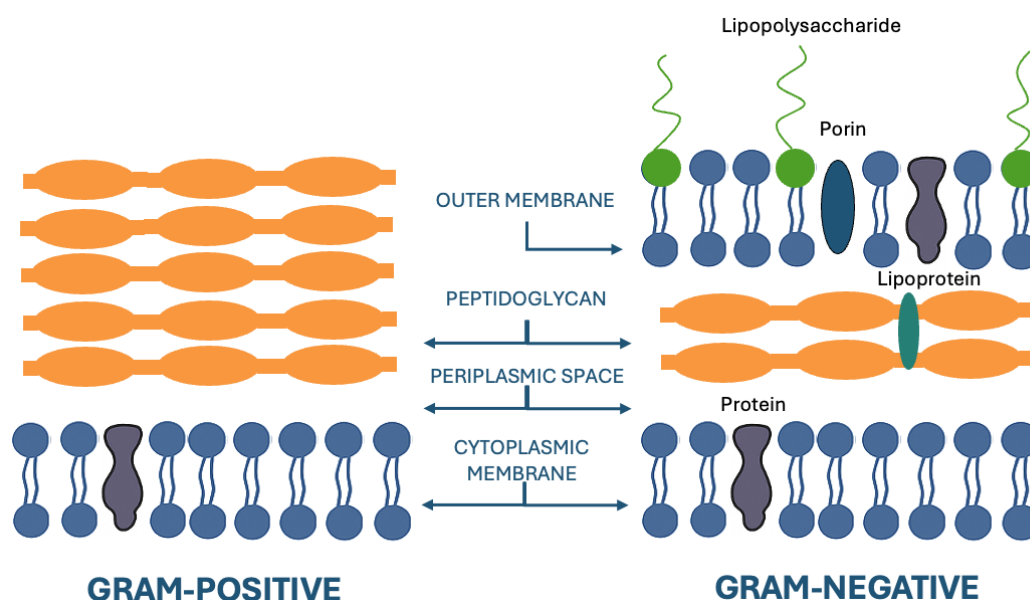


Figure 1. Schematic of key structural components in Gram-positive and Gram-negative cell walls

The biosorption of heavy metals by bacterial cells involves various attachment mechanisms, including ion exchange, physisorption, cytoplasmic accumulation, complexation, and both intra- and extracellular precipitation [23]. Usually, no single mechanism can fully explain metal uptake; rather, it often results from a combination of physicochemical interactions between metal ions and functional groups located on the bacterial cell surface [25]. The limiting transport pathways in bacterial uptake are usually film diffusion, surface adsorption, and intracellular diffusion across the cell membrane [26, 27]. As explained by Das et al. [24], all metal ions must first pass through the bacterial cell wall before reaching the cell membrane and cytoplasm. The bacterial cell wall is fundamental to the passive biosorption of heavy metals, particularly in Gram-negative species. Key structural components involved in metal binding include lipopolysaccharides containing -PO_4^{3-} groups, outer membrane proteins, and periplasmic peptidoglycan with -COOH and -NH_2 groups, as well as phospholipids also bearing -PO_4^{3-} functionalities [24, 28]. Additionally, membrane-associated proteins within the periplasm contribute -COOH and -NH_2 groups to the binding process [28]. In strains capable of forming biofilms, the secretion of extracellular polymeric substances (EPS), which is rich in -OH , -COOH , -NH_2 , and -PO_4^{3-} groups, further enhances the biosorption capacity by providing additional binding sites for metal ions [24, 29].

Despite the considerable advances over the past two decades, a critical gap remains in quantitatively understanding how different mechanisms contribute to the overall heavy metal uptake. To address this gap, this study introduces a structured protocol for elucidating both transport and attachment mechanisms in biosorption systems using viable *Pseudomonas aeruginosa* as a model biosorbent. By integrating kinetic modelling, equilibrium simulations, and both independent and sequential desorption analyses, the protocol distinguishes among physical adsorption, ion exchange, complexation, and intracellular diffusion pathways. The approach not only confirms the relevance of key sorption mechanisms but also quantifies their relative contributions to metal uptake. This level of mechanistic insight is essential for optimising biosorbent selection, improving process efficiency, and scaling treatment systems for real-world applications. Additionally, the protocol is adaptable to other biosorbents and contaminants, thus broadening its applicability as a tool for advancing adsorption development.

3. Materials and Methods

3.1. Growth and Harvesting of Bacterial Biomass

For the uptake of heavy metal ions, a pure strain of *Pseudomonas aeruginosa* was procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterium was cultivated in nutrient broth (DIFCO BACTO) at 30 °C for 24 h. Upon completion of the incubation, cells were separated from the growth medium by centrifugation at 6,300 rpm for 10 min using an IEC CENTRA MP4 centrifuge. The harvested biomass was subsequently washed three times with sterile 0.9% (w/v) physiological saline solution. To estimate the dry mass of the biomass, 5 mL aliquots of the washed *Pseudomonas aeruginosa* suspension were transferred to pre-weighed crucibles and dried in a hot-air oven at 80 °C for 24 h. Samples were then cooled in a desiccator for 30 min and re-weighed. Drying and weighing were repeated at 4-hr intervals until a constant mass (variation < 0.002 g) was achieved. The resulting average dry biomass concentration was 6.0 g/L. The biomass concentration of *Pseudomonas aeruginosa* was estimated by measuring the optical density at 600 nm with a Milton Roy Spectronic 601 spectrophotometer, and the corresponding values were determined using the calibration curve shown in Figure 2.

3.2. Preparation of Solutions and Analytical Methods

All reagents and chemicals used in this study were of analytical grade. Ethylenediaminetetraacetic acid (EDTA), hydrochloric acid, and other required chemicals were obtained from Merck (Darmstadt,

Germany). Stock solutions of lead(II) nitrate [$\text{Pb}(\text{NO}_3)_2$] and copper(II) sulfate pentahydrate [$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$] were prepared using double-distilled water produced by a Corning Mega Pure system, with a conductivity below $5 \mu\text{S}/\text{cm}$. Quantitative analysis of Cu(II) and Pb(II) ion concentrations in solution was performed using a flame atomic absorption spectrophotometer (AAS), model 3030B (Perkin-Elmer). Throughout all experimental procedures, the pH was maintained at 4.5-5.5 using a 0.01 M acetate buffer. pH values were measured with a calibrated digital pH meter (Accumet Research AR10, Fisher Scientific, USA).

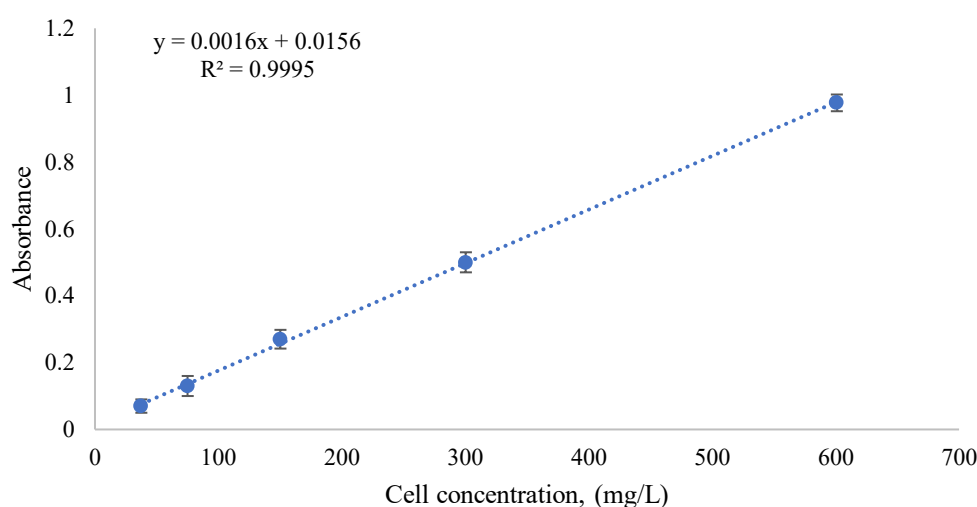


Figure 2. Calibration curve to correlate the optical density of *Pseudomonas aeruginosa* cells

3.3. Batch Biosorption Studies

All experiments were performed in triplicate, and the reported values represent mean $\pm$ standard deviation (SD). Data variability was assessed by calculating the SD of the three independent replicates. All media, glassware, and solutions were sterilised by autoclaving. Solution volumes were controlled within ± 0.5 mL. Metal ion uptake kinetics were examined under batch conditions using a biosorbent dosage of 1.0 g/L. Each batch system was prepared by spiking the reaction mixture with 50 mg/L of the target metal ion, followed by agitation at 250 rpm (SK-330-Pro Orbital Digital Shaker) for up to 60 min. After biosorption, the biomass was separated by centrifugation at 6300 rpm for 10 min. Equilibrium studies were conducted at room temperature (26 ± 1 °C) by varying the initial metal concentration from 10.0 to 100.0 mg/L. The solid-to-liquid ratio (S/L ratio) is the mass of biosorbent (solid phase) used relative to the volume of desorbing solution (liquid phase) [30]. Desorption experiments were performed in triplicate using a solid-to-liquid (S/L) ratio of 1.0 g L^{-1} , corresponding to 1.0 g of metal-loaded biosorbent in 50 mL of desorbing solution. The separated biomass was then contacted with an equal volume of 0.1 M HCl, 0.1 M EDTA, 0.1

M CaCl₂, or distilled water, and the mixtures were agitated for 90 min. Distilled water was used to remove weakly bound metal ions, as such release is characteristic of physical sorption [31]. Calcium chloride was included to evaluate the role of ion exchange; Pan et al. [32] reported that 0.1 M CaCl₂ effectively desorbed Sr²⁺ from *Aspergillus niger*. Ethylenediaminetetraacetic acid (EDTA, 0.1 M) was used to test the contribution of complexation, consistent with the findings of Puebla et al. [33], who demonstrated that this chelating agent released chemisorbed heavy metals bound to activated sludge. Before commencing the desorption trials, hydrochloric acid was tested for its ability to destroy *Pseudomonas aeruginosa* cells. Actively growing cultures were exposed to several acid concentrations to identify the lowest concentration causing complete loss of cellular integrity. After treatment, samples were neutralised and plated on nutrient agar to assess viability. No colony formation was observed after 48 h of incubation at 37 °C in the 0.1 M HCl treatment, confirming irreversible loss of viability and complete cellular destruction.

3.4. Kinetic and Equilibrium Modelling

Experimental kinetic data were analysed using several kinetic models, including the pseudo-first-order, pseudo-second-order, intraparticle diffusion, and diffusion-chemisorption models (Table 1). The equilibrium data were modelled using the Langmuir, Freundlich, and Sips isotherms (Table 2). The model's performance and fit quality were evaluated using the coefficient of determination (R²), relative percentage error (RPE), mean percentage standard deviation (MPSD), and the HYBRID error function (Table 3).

Table 1. Kinetic models

Model Name	Format	Equation	Equation No.	Reference
Lagergren pseudo-first-order model	Non-linear	$q_t = q_e(1 - \exp^{-K_{PFO}t})$	(1)	[34]
Pseudo-second-order	Non-linear	$q_t = \frac{K_{PSO}q_e^2t}{1 + K_2q_et}$	(2)	[35]
	Initial rate	$h = (K_{PSO})q_e^2$	(3)	
Diffusion-chemisorption	Non-linear	$q_t = \frac{q_eK_{DC}t^{0.5}}{q_e + K_{DC}t^{0.5}}$	(4)	[36, 37]
	Initial rate	$k_i = \frac{K_{DC}^2}{q_e}$	(5)	
Weber and Morris intraparticle diffusion model	Linear	$q_t = K_{ID}\left(t^{\frac{1}{2}}\right) + c_{ID}$	(6)	[38]

Table 2. Equilibrium models

Model Name	Format	Equation	Equation No.	Reference
Langmuir	Non-linear	$q_e = \frac{q_m K_L C_e}{1 + K_L C_e}$	(7)	[39]
Freundlich	Non-Linear	$q_e = K_F C_e^{1/n_F}$	(8)	[40]
Sips	Non-Linear	$q_e = \frac{q_{m-s} (\alpha_s C_e)^{n_s}}{1 + (\alpha_s C_e)^{n_s}}$	(9)	[41]

Table 3. Error functions

Error Functions	Expression	Equation No.
Relative Percent Error (RPE)	$RPE\% = \frac{1}{N} \sum_{i=1}^N \left[\frac{(q_{e_i})_{pred} - (q_{e_i})_{exp}}{(q_{e_i})_{exp}} \right] * 100$ where, N is the number of experimental points.	(10)
Marquardt's Percent Standard Deviation (MPSD)	$MPSD = 100 \sqrt{\frac{1}{N-P} \sum_{i=1}^N \left[\frac{(q_{e_i})_{exp} - (q_{e_i})_{pred}}{(q_{e_i})_{exp}} \right]^2}$ where, N is the number of experimental points and P is the number of parameters in the regression model.	(11)
The Hybrid Error Function (HYBRID)	$HYBRID = \frac{100}{N-P} \sum_{i=1}^N \left[\frac{((q_{e_i})_{exp} - (q_{e_i})_{pred})^2}{(q_{e_i})_{pred}} \right]$ where, N is the number of experimental points and P is the number of parameters in the regression model.	(12)
R^2	$R^2 = \frac{\sum_{i=1}^N ((q_{e_i})_{exp} - q_{e_{exp,mean}})^2 - \sum_{i=1}^N ((q_{e_i})_{exp} - (q_{e_i})_{pred})^2}{\sum_{i=1}^N ((q_{e_i})_{exp} - (q_{e_i})_{pred})^2}$ where N is the number of experimental points.	(13)

4. Results and Discussions

4.1. Kinetic Analysis

Contact time plays a crucial role in determining biosorption efficiency. It can influence the rate of metal uptake, the overall adsorption capacity, and can provide insight into the underlying transport mechanisms and equilibrium behaviour [42]. Four kinetic models, presented in Tables 1 and S4, were applied to fit the experimental data, and the model accuracy was assessed using error functions listed in Table 3. The pseudo-first-order, pseudo-second-order, and diffusion-chemisorption models all produced

high coefficients of determination, each exceeding 0.95. The pseudo-second-order model provided the best fit for Pb(II) biosorption, while Cu(II) was slightly better represented by the pseudo-first-order model.

Nevertheless, the pseudo-second-order model was selected for subsequent analysis due to its overall consistency. As shown in Figure 3, the effect of contact time revealed a two-stage biosorption pattern for both metal ions. The initial slope characterised the rapid initial uptake, followed by a slower approach to equilibrium. Cu(II) exhibited significant uptake within the first 10 min, while Pb(II) reached a similar phase within 30 min. Vimalnath et al. [43] reported a similar kinetic profile for the biosorption of Pb(II) by *Pseudomonas aeruginosa*. Consequently, as observed in Table 4, Pb(II) exhibited a lower initial and overall sorption rate, as determined by the pseudo-second-order model, when compared to that of Cu(II) biosorption. Equilibrium was generally attained within 60 min of the reaction. The better fit of the pseudo-first-order model for Cu(II) may have likely arisen from intrinsic differences in the physicochemical characteristics of the two cations. Cu(II) has a smaller ionic radius (0.73 Å) and a more strongly bound hydration shell, which may have led to slower dehydration and weaker surface complexation than Pb(II).

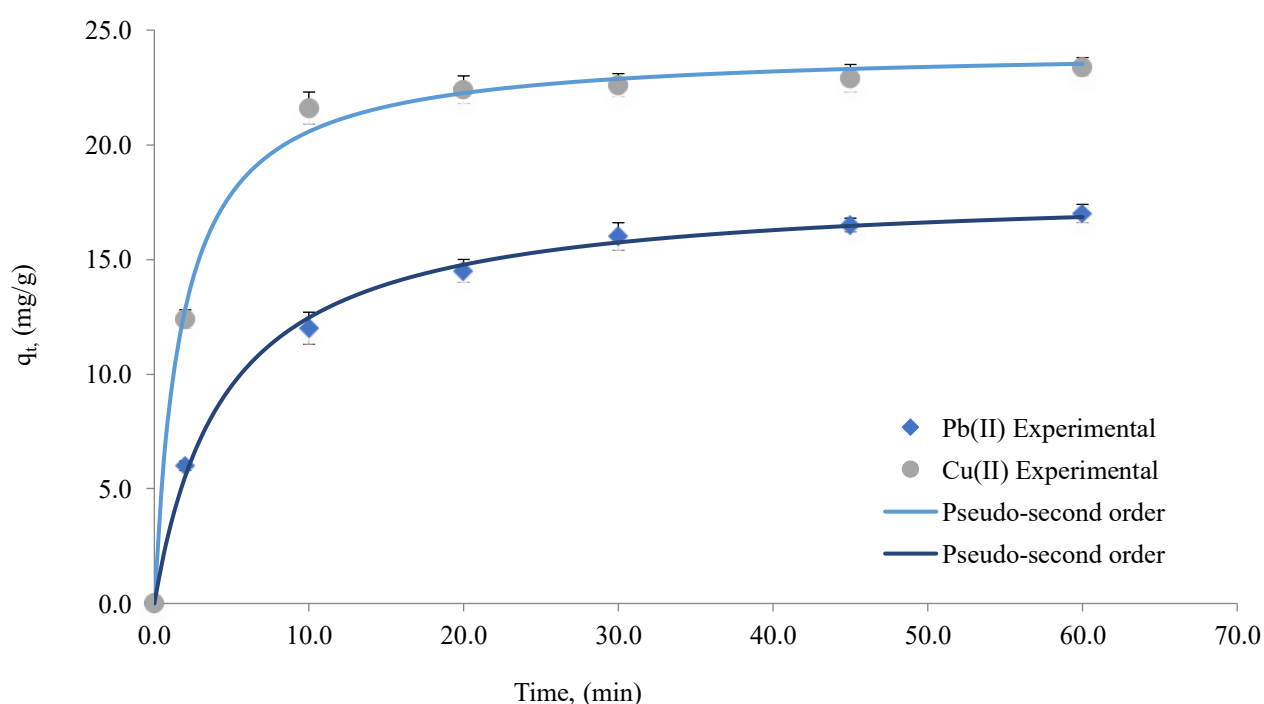


Figure 3. Pseudo-second-order model fitting to experimental data for Cu(II) and Pb(II)

Consequently, Cu(II) sorption proceeded mainly through rapid diffusion and reversible outer-surface interactions, which the PFO model well describes. In contrast, Pb(II) has a larger ionic radius (1.19 Å) and lower hydration energy, facilitating partial dehydration and stronger inner-sphere coordination with

functional groups on the cell surface. Such chemisorption reactions are consistent with pseudo-second-order (PSO) kinetics. Surface-site heterogeneity may also favour Cu(II) association at low-affinity outer sites, while Pb(II) binds preferentially to high-affinity inner sites characteristic of chemisorption. However, as noted by Ting and Mittal [44], classical kinetic and isotherm models were initially formulated for ideal gas-solid or liquid-solid systems, yet remain useful for describing complex biosorption phenomena. Nevertheless, mechanistic interpretations should be made with caution, as good statistical fits may reflect mathematical conformity rather than definitive physicochemical pathways.

Table 4. Kinetic model parameters for Cu(II) and Pb(II) biosorption by *Pseudomonas aeruginosa*

Model	Parameters	Cu(II)	Pb(II)
Pseudo-first-order	q_e , (mg/g)	22.69	16.20
	K_{PFO} , (1/t)	0.39	0.15
	R^2	0.9977	0.9794
	RPE	1.61	8.63
	MPSD	2.32	15.54
	HYBRID	1.18	17.99
Pseudo-second-order	q_e , (mg/g)	24.22	18.13
	K_{PSO} , (g/mg·t)	0.02	0.01
	h , (mg/g·t)	13.66	3.99
	R^2	0.9967	0.9976
	RPE	2.07	3.29
	MPSD	3.18	5.18
Intraparticle diffusion	c_{ID} , (mg/g)	6.81	2.75
	K_{ID} , (mg/g·t ^{0.5})	2.68	2.16
	R^2	0.7301	0.8925
	RPE	40.79	27.00
	MPSD	55.92	38.76
	HYBRID	574.05	157.42
Diffusion-chemisorption	q_e , (mg/g)	28.92	27.05
	K_{DC} , (mg/g·t ^{0.5})	19.05	6.51
	k_i , (mg/g·t)	12.54	1.57
	R_{DC} , (-)	0.196	0.537
	R^2	0.9819	0.9924
	RPE	5.36	4.31
	MPSD	8.36	7.82
	HYBRID	11.63	4.54

4.2. Variation in Solution Conductivity

In this experiment, the electrical conductivity (EC) of the solution was adjusted using potassium nitrate (KNO_3), which dissociates completely to yield K^+ and NO_3^- ions, thereby increasing the total ionic content of the solution without introducing complexing species. Biosorption experiments, using Cu(II) as a model cation, revealed that both the capacity and rate of metal uptake by *Pseudomonas aeruginosa* remained relatively stable up to an EC of 4.6 mS/cm (Figures 4 and 5). This suggests a preferential affinity at the cell surface of *Pseudomonas aeruginosa* for the divalent Cu(II) ions over that of the monovalent K^+ ions. However, at higher conductivities, a marked reduction in performance was observed. At an EC of 15.0 mS/cm, Table 5 shows that the adsorption capacity decreased by approximately 30% and the reaction rate decreased by 10%. This observed reduction in biosorption efficiency with increasing conductivity is attributed primarily to electrostatic screening and ion competition [45]. Elevated concentrations of K^+ ions in solution can lead to competitive adsorption [46]. Although K^+ has a lower affinity for the biosorbent compared to Cu(II) , its abundance can lead to partial occupancy or blockage of active binding sites. From a kinetic standpoint, the masking of the bacterial surface charge by background ions diminished the mass transfer driving force, thereby reducing the mobility of Cu(II) ions toward the surface and slowing the rate of attachment. This led to prolonged equilibration times and lower overall uptake.

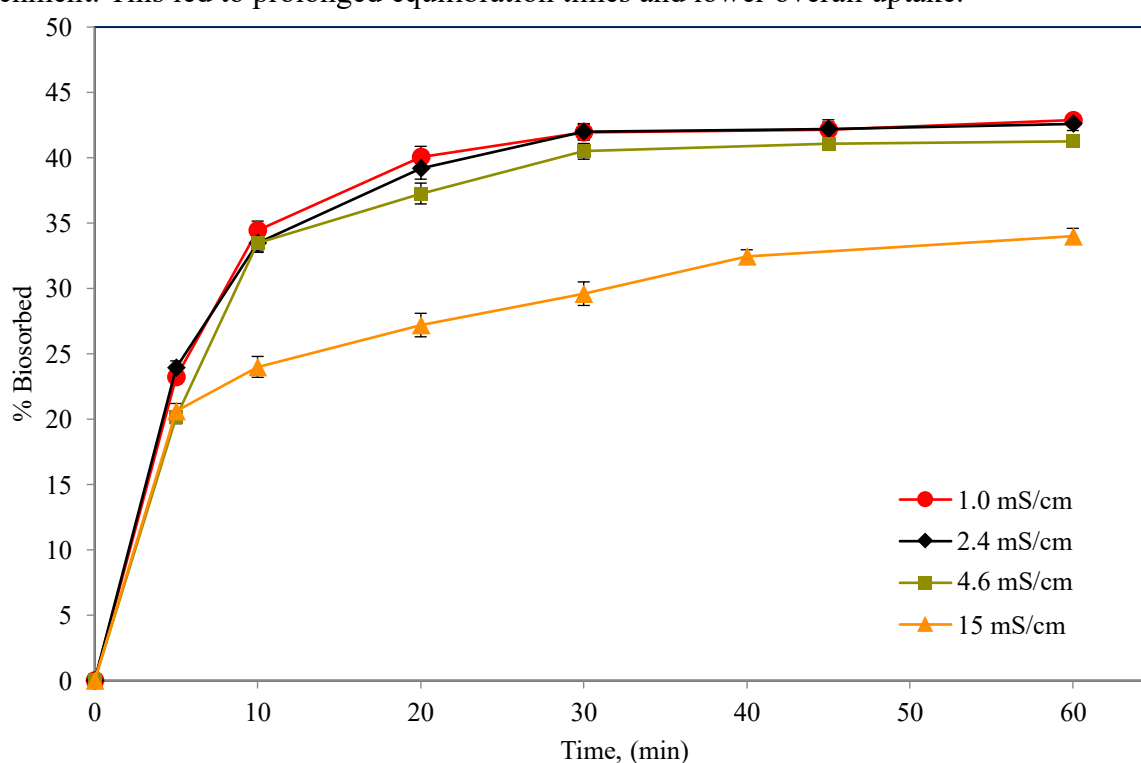


Figure 4. Kinetic plot showing the effect of solution conductivity on Cu(II) biosorption by *Pseudomonas aeruginosa*

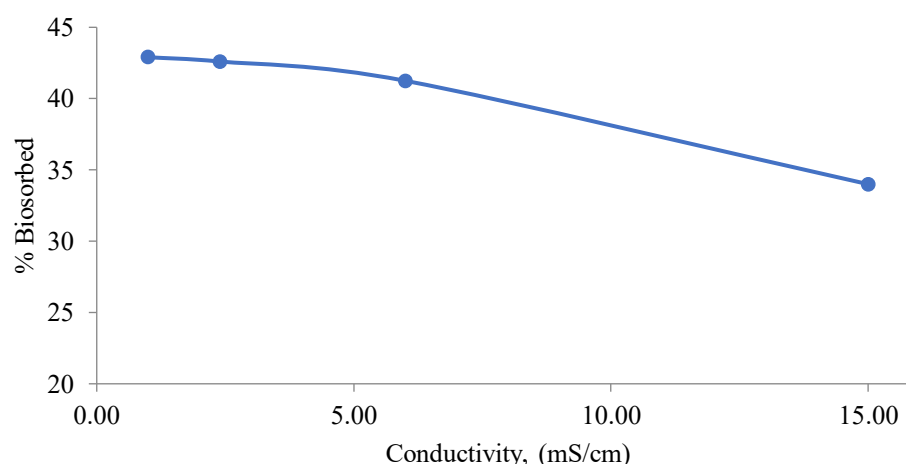


Figure 5. Variation in biosorption percentage with increasing conductivity for Cu(II) uptake by *Pseudomonas aeruginosa*

4.3. Effect of Solution pH on Cu(II) Biosorption

The impact of pH on the biosorption performance of viable *Pseudomonas aeruginosa* was assessed over a pH range of 4.5–5.5 using Cu(II) as the model cation. The upper limit of 5.5 was selected to minimise the risk of $\text{Cu}(\text{OH})_2$ precipitation, which could otherwise distort the interpretation of the biosorption data. The lower limit of 4.5 was chosen to preserve microbial viability and prevent potential cell death under highly acidic conditions. Although direct confirmation of cell viability (e.g., by live/dead staining or post-exposure plating) was not performed, viability was assumed to be maintained over the 60-minute contact period. The approach to equilibrium typically began within 30 min of agitation, with the lowest metal removal observed at pH 4.5 (Figure 6). This also corresponded to a reduced initial and overall sorption rate as shown in Table 5. Established knowledge of *Pseudomonas aeruginosa* surface chemistry indicates that (i) a decrease in pH or a reduction in anionic functional groups would suppress ion exchange and favour complexation-type retention, whereas (ii) an increase in surface negative charge, through deprotonation at higher pH or enhanced expression of acidic polysaccharides, would promote ion-exchange interactions. Thus, the decline in performance at lower pH was likely due to the protonation of functional groups on the bacterial surface, leading to a net positive surface charge [47]. This positively charged cell surface may have repelled the Cu(II) ions, thereby reducing electrostatic attraction.

Furthermore, competition from excess H^+ ions for active binding sites may have further suppressed metal uptake [48]. Similar results have been reported by Miftah [49] for the adsorption of Cd(II) by *Ochrobactrum intermedium*. Maximum Cu(II) removal occurred at pH 5.5, consistent with findings by

González et al. [50] for *Pseudomonas aureofaciens*. At pH values above 5.0, the deprotonation of surface functional groups increases the availability of negatively charged sites, enhancing electrostatic attraction between the bacterial surface and Cu(II) ions. Further, functional groups on *Pseudomonas aeruginosa*, such as -COO^- and -PO_4^{2-} , are known to form stable complexes or coordinate to Cu(II) ions, thereby promoting biosorption [51].

Table 5. Kinetic parameters as a function of solution pH and conductivity for Cu(II) biosorption

Parameter	q_m , (mg/g)	K_{PSO} , (g/mg·t)	h , (mg/g·t)	R^2
pH				
pH 5.5	24.22	0.023	13.659	0.9967
pH 5.0	23.56	0.024	13.359	0.9994
pH 4.5	21.83	0.018	8.782	0.9996
Conductivity				
1.0 mS/cm	25.21	0.009	5.754	0.9927
2.4 mS/cm	24.63	0.009	5.682	0.9966
4.6 mS/cm	24.33	0.008	4.762	0.9868
15.0 mS/cm	17.40	0.014	4.279	0.9884

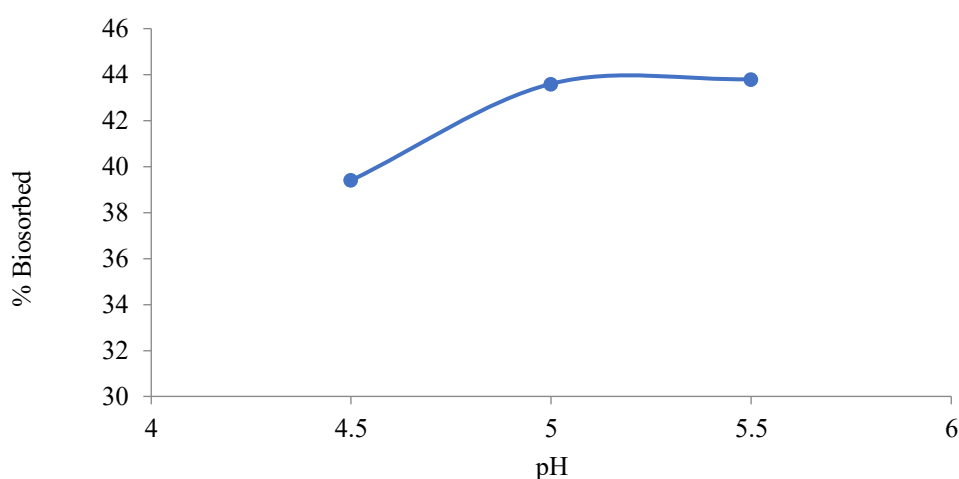


Figure 6. Effect of solution pH on Cu(II) biosorption by *Pseudomonas aeruginosa*

4.4. Equilibrium Studies

The experimental data for the biosorption of Cu(II) and Pb(II) by *Pseudomonas aeruginosa* were modelled by non-linear regression using the Langmuir, Freundlich, and Sips isotherms (Tables 2 and S6). The coefficient of determination determined the fit accuracy. Table 6 presents the model parameters and calculated error. Based on observations, the best fit to the data was achieved by the Sips isotherm, followed by the Langmuir isotherm, and finally the Freundlich isotherm (Figure 7). This was also supported by the

error values presented in Table 6. The Sips isotherm, also known as the Langmuir-Freundlich isotherm, is a hybrid adsorption model that combines the characteristics of both the Langmuir and the Freundlich isotherms. The model is particularly useful for describing adsorption onto heterogeneous surfaces. At low adsorbate concentrations, the Sips model reduces to the Freundlich isotherm. While at high concentrations, the model converges to the Langmuir isotherm, indicating monolayer adsorption onto a finite number of sorption sites [52].

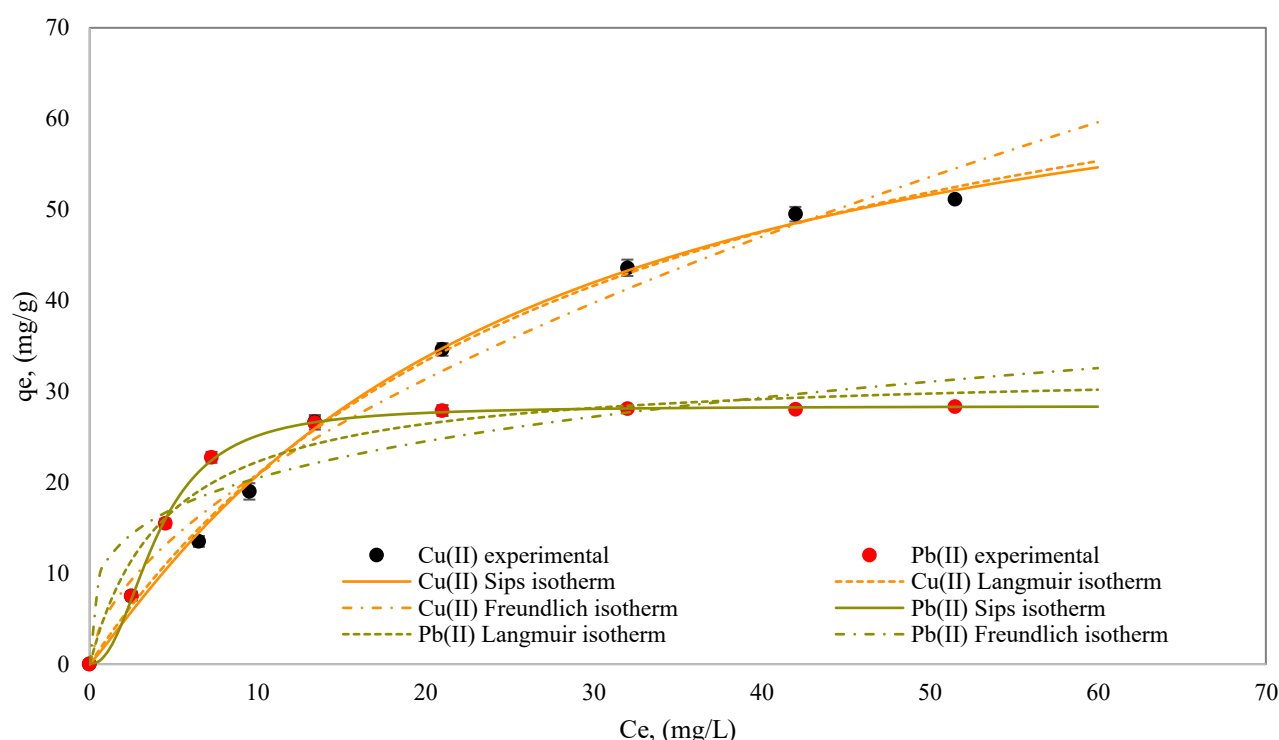


Figure 7. Evaluation of equilibrium isotherm model simulations against experimental biosorption data for Cu(II) at pH 5.5 and Pb(II) at pH 5.0

Additionally, the Sips heterogeneity parameter (n_s) offers insight into surface characteristics. Values close to 1 reflect homogeneity, values less than 1 suggest heterogeneity, and values greater than 1 indicate cooperative adsorption. In this study, n_s values were 1.10 for Cu(II) and 2.25 for Pb(II), suggesting positive cooperativity in the sorption process, i.e., where initial metal biosorption may facilitate further uptake. This may have resulted from intermolecular interactions, such as van der Waals forces, hydrogen bonding, or electrostatic attractions, between biosorbed species and active sites on the biosorbent surface. The Langmuir model also demonstrated a strong fit, with predicted monolayer adsorption capacities of 82.48 mg/g for Cu(II) and 32.50 mg/g for Pb(II). Zhang et al. [53] reported maximum biosorption capacities of 72.5 mg/g for Cu(II) at pH 6.0 and 94.2 mg/g for Pb(II) at pH 7.0 using a metal-resistant strain of

Pseudomonas aeruginosa isolated from soil. The markedly higher Pb(II) uptake may be attributed to both the strain's metal-resistance traits and the elevated pH, where concurrent precipitation and biosorption are likely to occur. A comparative summary of Cu(II) and Pb(II) biosorption capacities reported for bacterial sorbents, inclusive of the data generated in this study, is presented in Table S7. The Cu(II) uptake observed for the *Pseudomonas aeruginosa* strain compared favourably to values previously reported in the literature for other bacterial biosorbents. In contrast, the Pb(II) uptake in this study was comparatively lower. Based on scrutiny of the literature, similar experimental conditions were observed; thus, this difference is more plausibly linked to the intrinsic physiological and surface-chemical characteristics of the microorganism, which can influence metal selectivity and binding affinity.

Table 6. Parameters and error estimates from Langmuir, Freundlich and Sips models for Cu(II) and Pb(II)

Isotherm	Parameters	Cu(II)	Pb(II)
Langmuir	q_{m-L}	82.48	32.50
	K_L	0.03	0.22
	R^2	0.9970	0.9599
Freundlich	K_F	5.44	11.29
	n_F	1.71	3.86
	R^2	0.9858	0.8817
Sips	q_{m-S}	74.20	28.39
	α_S	0.04	0.25
	n_S	1.10	2.25
	R^2	0.9973	0.9995

4.5. Transport Mechanisms of Biosorption

To elucidate the underlying transport mechanisms governing the biosorption process, both the Weber and Morris intraparticle diffusion model and the Sutherland diffusion-chemisorption (D-C) model were applied to the kinetic data for Cu(II) and Pb(II) biosorption. Using the Weber and Morris model, plots of qt vs. $t^{0.5}$ for Cu(II) and Pb(II) reveal multilinear trends (Figure 8). This indicates the presence of multiple rate-limiting steps [54]. Notably, the curves did not intersect the origin. According to Ofomaja [55], a larger intercept indicates a stronger boundary-layer effect, implying that, in this instance, film diffusion had a stronger influence on Cu(II) biosorption than on Pb(II). As described by Wu et al. [56], the Weber-Morris model characterises biosorption as progressing through three phases: (i) rapid external surface adsorption, (ii) a slower intraparticle diffusion phase, and (iii) a final equilibrium stage, where diffusion transitions from macropores to micropores.

In the current study, both Cu(II) and Pb(II) biosorption curves displayed multiple distinct linear segments. This behaviour corresponded to an initial rapid uptake followed by a more gradual diffusion step, likely associated with slower intracellular transport across the bacterial membrane. Equilibrium was attained during the second biosorption phase for both metals. *Pseudomonas aeruginosa* displayed a higher biosorption capacity for Cu(II) (Table 5) and a faster overall uptake rate (Table 4). The rapid initial sorption of Cu(II) was further supported by the pseudo-second-order initial rate constant, h (Table 4), which was more than three times greater than that of Pb(II). For Pb(II), intracellular diffusion dominated during the final 30 min of the 60-min reaction, whereas for Cu(II), this phase extended over approximately 50 min. Additionally, the slope of the Pb(II) diffusion plot (0.4397) was slightly higher than that of Cu(II) (0.361), suggesting marginally faster intraparticle transport for Pb(II) under the studied conditions.

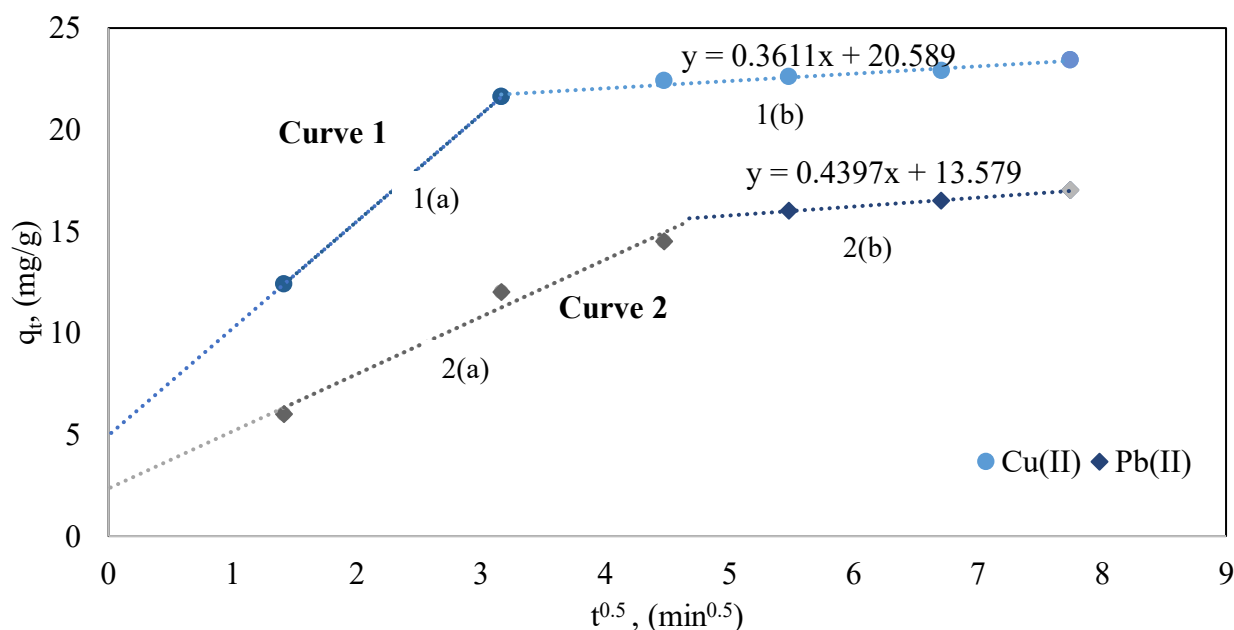


Figure 8. Experimental data plotted according to the Weber and Morris diffusion model for Cu(II) and Pb(II)

In adsorption systems, the rate-limiting mechanism may emerge as distinct stages at different points during the process. However, multiple mechanisms can also act concurrently or overlap in the background, collectively slowing the overall adsorption rate. To further investigate the role of intraparticle diffusion, interpreted here as intracellular or membrane-associated transport, the diffusion-chemisorption (D-C) model was applied to the kinetic data. This model incorporates the solid-phase mass transfer index (R_{DC}) [57], which was calculated using the relationship presented in Eq. (14), where the parameters q_e and K_{DC} are obtained from regression of the experimental data from Table 4 and t_{ref} (min) is the longest experimental time in an adsorption system (60 min in this study).

$$R_{DC} = \frac{q_e}{K_{DC} t_{ref}^{0.5}} \quad (14)$$

The D-C model also enables the generation of a characteristic curve using Eq. (15):

$$Q_t = \frac{T [1 + R_{DC}]}{T + R_{DC}} \quad (15)$$

Using this formulation, the theoretical or standard characteristic curve can be constructed using Eq. (15) by applying fixed R_{DC} values of 0.01, 0.1, 1 and $\gg 1$ (Figure 9). The values of Q_t can be calculated for any given value of T between 0 and 1. The experimental characteristic curve is then overlaid by solving Eq. (15) for values of Q_t and T , where Q_t and T are given by Eqs. (16) and (17), and q_{ref} (mg/g) represents the amount of adsorption at time t_{ref} . The detailed procedure is outlined in Tables S1-S4.

$$Q_t = \frac{q_t}{q_{ref}} \quad (16)$$

$$T = \frac{t^{0.5}}{t_{ref}^{0.5}} \quad (17)$$

The D-C model exhibited strong agreement with the kinetic data, yielding coefficients of determination (R^2) of 0.9819 for Cu(II) and 0.9924 for Pb(II). The corresponding values of the intraparticle diffusion influencing factor, represented by the solid-phase mass transfer index (R_{DC}), were 0.196 and 0.537 for Cu(II) and Pb(II), respectively (Table 4). Both values fall within the range of $0.1 \leq R_{DC} < 1$, which, according to Table 7, denotes moderate control by intraparticle diffusion. Notably, the higher R_{DC} value for Pb(II) suggests a greater role of intracellular diffusion in the overall biosorption process than for Cu(II). This interpretation was reinforced by the characteristic curves generated by the D-C model (Figure 9). It must be emphasised that the Weber and Morris model reported that for Cu(II), the duration of the intraparticle diffusion phase was longer than that of Pb(II). In contrast, the D-C model highlights a more pronounced and persistent role of intracellular diffusion during the overall Pb(II) biosorption. This could explain the more prolonged uptake behaviour observed for Pb(II), suggesting that, even when not acting as the rate-limiting step, intracellular diffusion may have continuously influenced the process in the background.

Table 7. R_{DC} index thresholds for assessing intraparticle diffusion control in adsorption kinetics

R_{DC} Value	Shape of Kinetic Curve	Influence of Intraparticle diffusion and chemisorption	Type
$R_{DC} \geq 1$	Slightly Curve	Significant Influence of Intraparticle Diffusion	I
$0.1 \leq R_{DC} < 1$	Moderately Curved	Moderate Influence of Intraparticle Diffusion	II
$0.01 \leq R_{DC} < 0.1$	Largely Curved	Low Influence of Intraparticle Diffusion	III
$R_{DC} < 0.01$	Pseudo-Rectangular	Insignificant Influence of Intraparticle Diffusion	IV

Note. R_{DC} = solid-phase mass transfer index

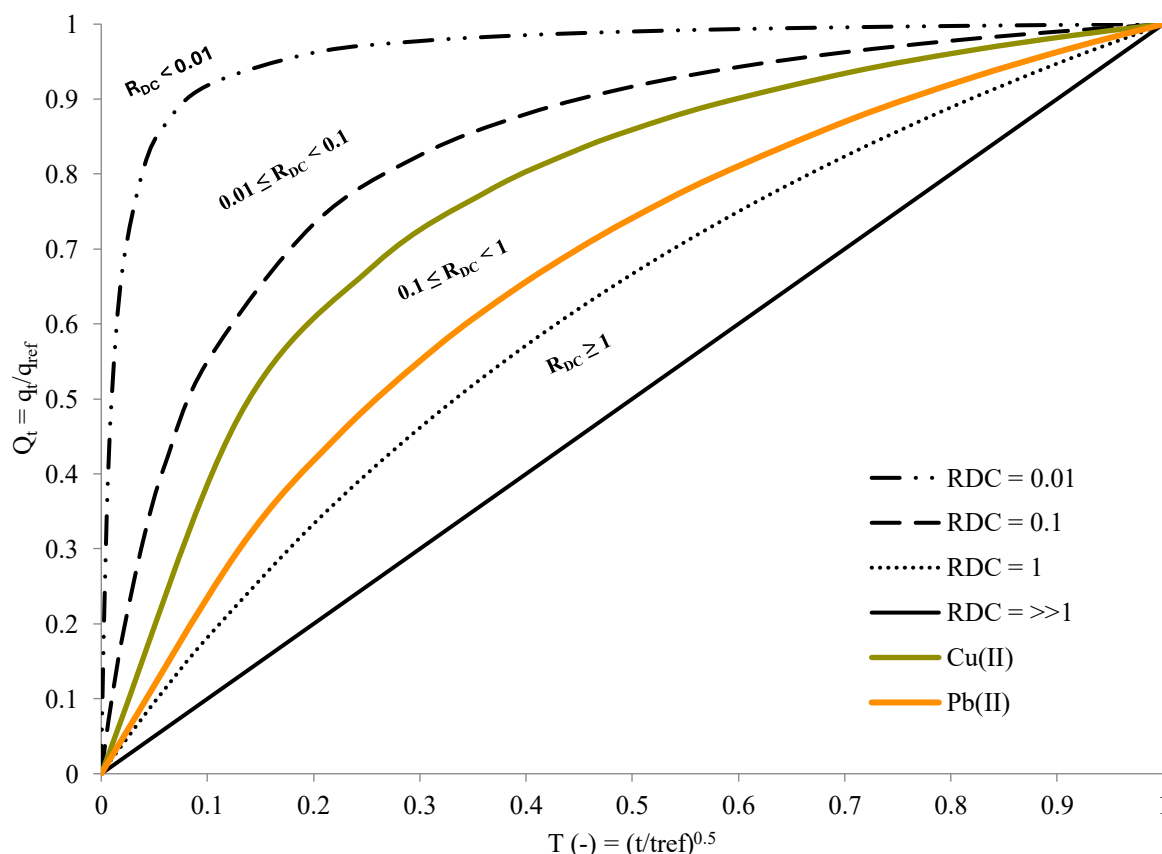


Figure 9. Characteristic curves of the diffusion-chemisorption model with Cu(II) and Pb(II) biosorption data by *Pseudomonas aeruginosa* overlaid

4.6. Elucidating Attachment Mechanisms

4.6.1. Elution Studies Using Individual Eluents

Desorption analysis is essential for assessing the practical applicability of a biosorbent, its metal-ion recovery potential, and the nature of the underlying adsorption mechanisms. As noted by Allard, et al. [58] sorption onto solid surfaces may proceed through three main mechanisms: (i) physical adsorption, characterized by its rapid and reversible nature and governed by non-specific van der Waals interactions; (ii) electrostatic attraction, which results from Coulombic forces between oppositely charged solute ions and the adsorbent surface and is similarly fast and reversible; and (iii) specific adsorption, where chemical bonding occurs at particular sites on the surface, often proceeding more slowly and exhibiting partial irreversibility due to the formation of strong surface complexes. In this study, metal-laden *Pseudomonas aeruginosa* was eluted with several solutions to elucidate the attachment mechanisms underlying the sorption of Cu(II) and Pb(II). The elution solutions selected were distilled water, 0.1 M calcium chloride (CaCl₂), 0.1 M hydrochloric acid (HCl), and 0.1 M ethylenediaminetetraacetic acid (EDTA).

Metal ions held by physical adsorption are readily released by a distilled water wash, due to the weak van der Waals interactions [59]. The release of 1.0% and 7.0% of Cu(II) and Pb(II) (Figures 10 and Figure 11), respectively, was recorded within the first 15 min of reaction (Figures 12 and Figure 13). The CaCl₂ wash introduces an excess of Ca²⁺ ions, which, through ion exchange, can compete with Cu(II) and Pb(II) for binding sites. This treatment released 47% of Cu(II) and 71% of Pb(II) ions within 20 min. This implied a significant contribution from electrostatic interactions and exchangeable binding sites. EDTA is a chelating agent that forms stable, soluble complexes with metal ions over a broad pH range, typically from pH 2 to 12 [60]. However, EDTA chelating efficiency begins to decline as it undergoes protonation at pH values below 4.0 [61]; thus, in these experiments, pH was maintained above 5.0. Elution with EDTA was achieved after 60 min of reaction, yielding 98% and 93% release of Cu(II) and Pb(II) ions, respectively. The HCl wash, due to its high proton concentration, was used to compromise bacterial integrity and release both surface-bound and intracellularly held metals. This treatment achieved complete desorption of 100% of Cu(II) and Pb(II) within the first 2 min of the reaction.

4.6.2. Sequential Elution

To further investigate the underlying attachment mechanisms, a sequential desorption approach was employed using multiple eluents. The same metal-loaded *Pseudomonas aeruginosa* biomass was subjected to successive washes with the four different desorbing agents in a fixed order: distilled water, calcium chloride (CaCl₂), ethylenediaminetetraacetic acid (EDTA), and finally hydrochloric acid (HCl). Each desorption step lasted 90 min, after which the biosorbent was recovered and reintroduced to the next eluent. This stepwise procedure allowed for the assessment of the specific contribution of each binding mechanism to overall metal uptake. The cumulative ion release across the four stages was compared with the desorption achieved by each eluent individually.

As illustrated in Figures 10 and Figure 11, the sequential treatment resulted in the complete (100%) recovery of the adsorbed metal ions. The desorption results indicate that CaCl₂ effectively released metal ions associated with both physisorption and ion exchange mechanisms, whereas EDTA and HCl were capable of releasing ions bound via physisorption, ion exchange, and complexation. The distilled water wash resulted in minimal desorption, approximately 1% for Cu(II) and 7% for Pb(II). The CaCl₂ wash mobilised 46% of Cu(II) and 64% of Pb(II), attributed to ion-exchange interactions. EDTA release of 51% of Cu(II) and 22% of Pb(II), affirming its role in disrupting both ion exchange and complexation bonds.

HCl released an additional 2% of Cu(II) and 7% of Pb(II), likely through intracellular release. Based on these observations, the adsorption of Cu(II) by *Pseudomonas aeruginosa* can be mechanistically attributed to approximately 1% physisorption, 46% ion exchange, 51% complexation, and 2% intracellular accumulation. Further, Pb(II) sorption appears to involve 7% physisorption, 64% ion exchange, 22% complexation, and 7% intracellular retention.

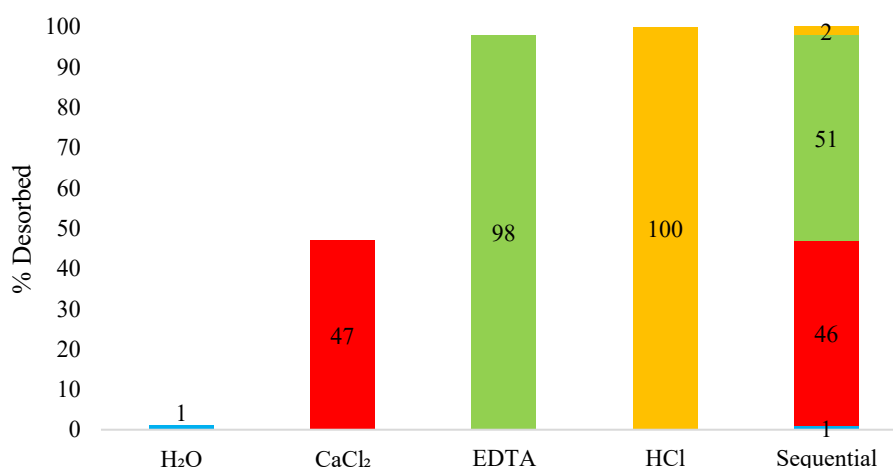


Figure 10. Cu(II) desorption from *Pseudomonas aeruginosa* using various eluents

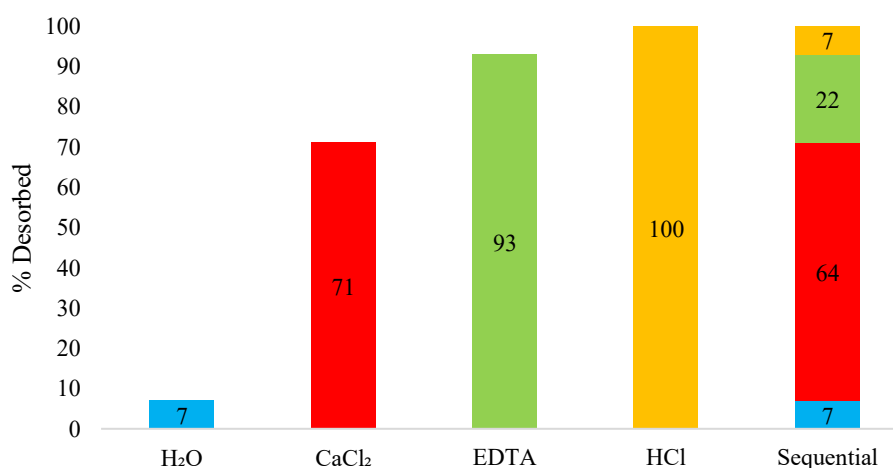


Figure 11. Pb(II) desorption from *Pseudomonas aeruginosa* using various eluents

Thermodynamically, this sequential desorption pattern (distilled H₂O → CaCl₂ → EDTA → HCl) represents an increasing energetic requirement for metal removal. Quantitatively, the CaCl₂ and EDTA steps together accounted for the majority of the sorbed metal for both Cu(II) and Pb(II). These results indicate that both metals are predominantly stabilised by higher-affinity interactions rather than by weak physical sorption. In particular, Pb(II) shows a greater contribution from the CaCl₂-released fraction, which

suggests that ion exchange at negatively charged sites is thermodynamically favoured for Pb(II). Further, Cu(II) shows a larger EDTA-released fraction, consistent with stronger site-specific coordination/chelation. Thus, the desorption sequence reflects an energetic hierarchy of binding. The relative magnitudes of the CaCl₂- and EDTA-released fractions provide quantitative support that ion-exchange-type interactions dominated Pb(II) uptake, while Cu(II) exhibited a greater contribution from complexation-driven binding.

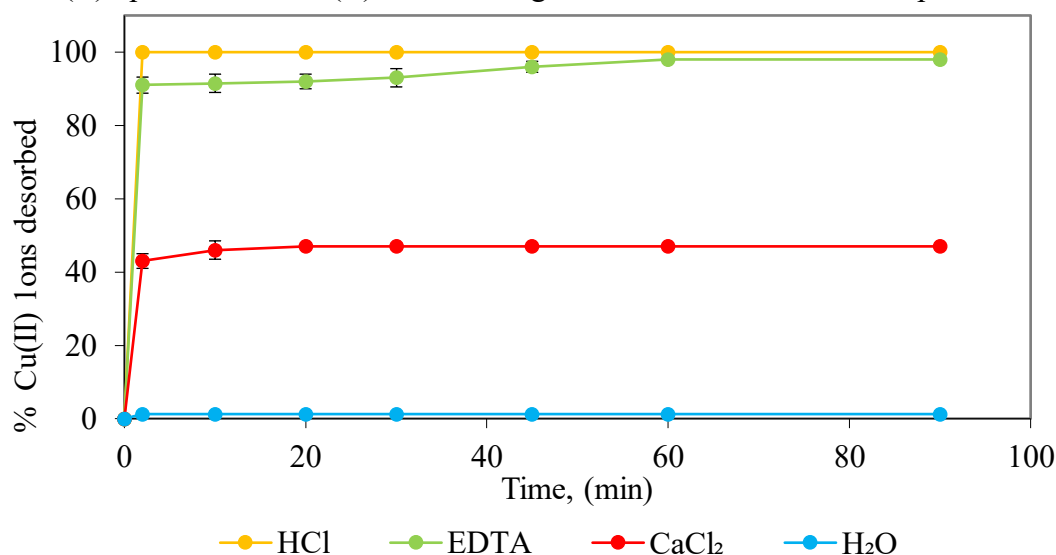


Figure 12. Desorption kinetics of Cu(II) release from *Pseudomonas aeruginosa* using various eluents

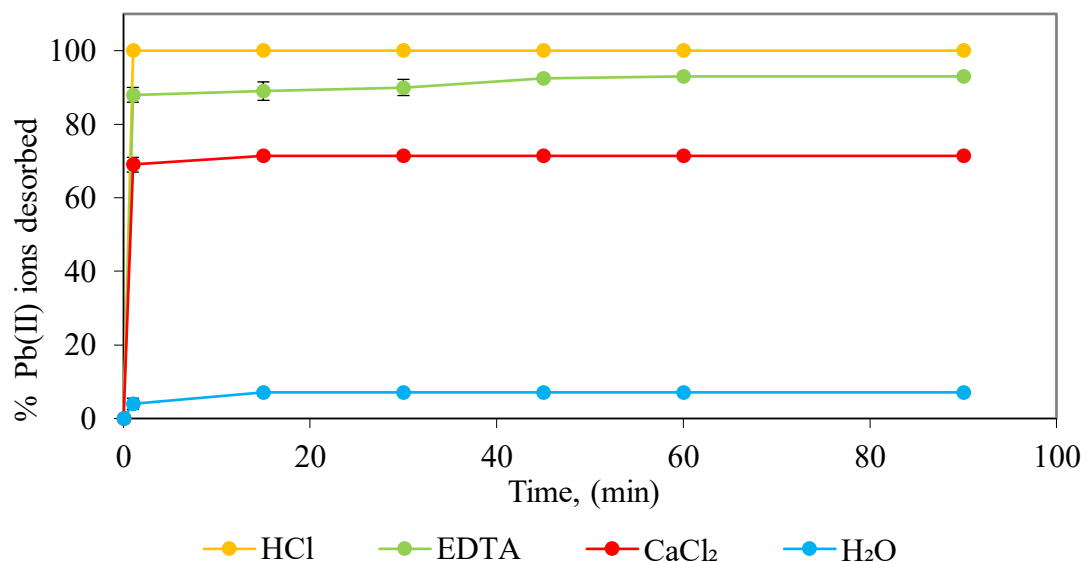


Figure 13. Desorption kinetics of Pb(II) release from *Pseudomonas aeruginosa* using various eluents

In many biosorption studies, attribution of binding mechanisms is inferred primarily from single-step desorption with one eluent, for instance, EDTA for complexation [33] or mineral acid for intracellular retention [62], or from qualitative FTIR peak shifts [63], without resolving how strongly different mechanisms contribute to the overall uptake. This approach can identify which interactions are plausible, but it rarely provides a quantitative breakdown of their relative importance. In contrast, the present protocol

applies both (i) individual desorption tests using distilled water, CaCl₂, EDTA, and HCl, and (ii) a structured sequential desorption of the same metal-loaded biomass with those eluents in series. The sequential step enables cumulative recovery of the adsorbed metal and allows the contributions of physisorption, ion exchange, surface complexation, and intracellular retention to be expressed as discrete percentages of total uptake. This level of mechanistic apportionment provides greater resolution than single-eluent methods and permits direct comparison between systems. Accordingly, the method not only identifies which mechanisms are active but also ranks their relative importance, a critical factor for process design and scale-up.

4.7. Limitations and Future Work

This study provides important insights into the mechanisms associated with Cu(II) and Pb(II) biosorption by *Pseudomonas aeruginosa*. Experimental conditions were restricted to room temperature (26 ± 1 °C). Broader investigations across environmental conditions, including thermodynamic analyses (ΔG° , ΔH° , ΔS°) and assessments of the effects of competing ions and organic matter, will further aid in process optimisation. The concentration range tested (10-100 mg/L) was suitable for contaminated effluents; however, future work should extend to both trace levels and higher levels to broaden applicability. This study was conducted under controlled laboratory conditions with relatively short contact times (≤ 60 min) and buffered media (pH 4.5–5.5), which helped preserve cellular viability and limit extensive EPS formation.

However, the potential influence of bacterial metabolic by-products, including extracellular polymeric substances and soluble organic ligands, was not directly quantified. These metabolites may contribute to metal complexation or modify surface-binding characteristics. Future investigations should therefore include quantitative analyses of extracellular metabolites, EPS composition, and metabolic activity under varying environmental conditions to refine the roles in diffusion and sorption mechanisms. Desorption experiments provided useful evidence of the dominant binding mechanisms, but future studies should confirm these interpretations through direct spectroscopic and microscopic analyses. Techniques such as FTIR, XPS, and SEM–EDX could help identify specific functional groups and metal-binding sites, thereby linking macroscopic desorption patterns to molecular-level interactions. It is also recognised that, as with all sequential desorption schemes, complete selectivity cannot be guaranteed at each stage, and

some residual metal fractions may persist until subsequent extractions. This overlap is inherent to biosorption systems, where multiple binding mechanisms often coexist at shared functional groups. The final HCl step, therefore, represents total recovery of the remaining metal content, combining contributions from residual complexed and intracellularly bound fractions.

5. Conclusions

This study presented a comprehensive protocol for elucidating the transport and attachment mechanisms involved in the biosorption of Cu(II) and Pb(II) by *Pseudomonas aeruginosa*. A combination of kinetic, equilibrium, and desorption experiments was used to develop the protocol that aids in differentiating the key biosorptive interactions. Kinetic analysis revealed a multiphase profile that comprised an initial rapid uptake due to surface biosorption, followed by a slower intracellular diffusion phase. Pb(II) was best represented by the pseudo-second-order model, while Cu(II) was best represented by both the pseudo-first- and the pseudo-second-order models. The diffusion-chemisorption (D-C) model provided additional insight, with R_{DC} values confirming moderate control by intracellular diffusion, especially for Pb(II). Equilibrium data fitted best to the Sips isotherm, indicating heterogeneous surface interactions and possible cooperative binding behaviour. Maximum monolayer adsorption capacities of 82.48 mg/g for Cu(II) and 32.5 mg/g for Pb(II) were observed at pH 5.5 and 5.0, respectively, comparable to values reported for other microbial biosorbents. Desorption studies using both individual and sequential elution approaches were used to expound the nature of the metal binding. Ion exchange and complexation were the dominant attachment mechanisms, while physisorption and intracellular accumulation contributed modestly. Desorption analysis revealed that for Cu(II) biosorption, physisorption, ion exchange, complexation, and intracellular accumulation accounted for 1%, 46%, 51% and 2%, respectively. For Pb(II), physisorption, ion exchange, complexation, and intracellular accumulation accounted for 7%, 64%, 22%, and 7%, respectively.

In summary, the study established an integrated kinetic-equilibrium-desorption framework that reliably elucidates both the transport and attachment mechanisms governing metal biosorption by *Pseudomonas aeruginosa*. The protocol outlined below consolidates the analytical approach used to differentiate mechanistic pathways and is intended to facilitate reproducibility and broader application. The developed framework also offers a practical tool for future research aimed at optimising microbial

biosorbents for heavy metal removal. For ease of application in future studies, the key elements of the protocol are summarised below.

Transport Mechanisms

- Film diffusion: Identified as the initial mass-transfer step by the Weber-Morris model; more pronounced for Cu(II) than for Pb(II).
- Initial external sorption: Confirmed by the early steep slope in the Weber-Morris plot and by the pseudo-second-order rate constant h ; Cu(II) exhibited an initial rate approximately three times higher than Pb(II).
- Intraparticle/intracellular diffusion: Represented the slower, rate-limiting stage. The Weber-Morris model defined the duration of this phase, while the diffusion-chemisorption (D-C) model quantified its influence through the R_{DC} parameter. R_{DC} values indicated moderate diffusion control, particularly for Pb(II).

Variations in agitation speed, particle size, or biomass concentration can alter the extent of diffusional limitation, which can be quantitatively evaluated using the D-C model.

Attachment Mechanisms

- Physisorption: Quantitatively identified through metal release in the distilled-water wash; consistent with pseudo-second-order kinetics and the Sips heterogeneity parameter (n_s).
- Ion exchange: Confirmed as the dominant mechanism by metal recovery in the CaCl_2 wash.
- Complexation: Evidenced by EDTA-induced desorption and supported by conformity to the pseudo-second-order model, indicating strong coordination with surface functional groups.
- Intracellular accumulation: Quantified through residual desorption with HCl, representing strongly bound or internalised metal ions.

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Data Availability Statement: The data supporting the findings of this study are included within the article and its supplementary materials. Additional processed data are available from the corresponding author upon reasonable request.

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Research Involving Human/Animal Participants: This article does not involve any studies conducted on human participants or animals. The study was conducted using bacterial cells under laboratory conditions.

Supplementary Material: The supplementary material associated with this article is available online and includes the detailed RDC calculation procedure, Tables S1–S7, Figure S1, and supplementary references.

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