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Heavy metals bioremediation: Cd^{2+}, Pb^{2+} and Zn^{2+} bioremoval by *Serratia marcescens* CCMA1010 in aqueous solution

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ABSTRACT

Microorganisms offer cost-effective and sustainable solutions for the bioremediation of toxic metals. S. marcescens CCMA1010 was investigated for its growth and metal removal potential in aqueous solution with Cd^{2+}, Pb^{2+}, and Zn^{2+}. The bacterium tolerated all three metals, with a minimum inhibitory concentration (MIC) ranking of $Zn^{2+} > Pb^{2+} > Cd^{2+}$. Remarkably, Pb^{2+} had minimal impact on growth compared to the control. A brown coloration in Pb^{2+} treatments suggested the bioprecipitation. FTIR analysis confirmed spectral changes linked to sulphate and phosphate groups, supporting the probability of this mechanism. No similar evidence was found for Cd^{2+} or Zn^{2+}. The bioremoval experiment was designed to construct a predictive model and identify the optimal conditions for maximum metal uptake (q), using a Rotatable Central Composite Design (RCCD) with 27 experimental runs, including factorial points, axial points, and three central points. The statistical modeling (P-value) showed that quadratic models for Cd^{2+} and Pb^{2+} uptake were significant ($p < 0.05$), with initial metal concentration as the main influencing factor. The p-value for Zn^{2+} (q) was not significant ($p > 0.05$). The F-values indicate that both models (Cd^{2+} and Pb^{2+}) were statistically significant, with only a 0.01% and 0.46% probability, respectively, that such high F-values could have occurred due to random noise. Variables such as pH and interactions between multiple metals were not statistically significant under the tested conditions. These results highlight S. marcescens CCMA1010 as a promising candidate for heavy metal bioremediation, particularly for lead removal through bioprecipitation pathways.

1. Introduction

The management of pollution, particularly that caused by toxic metals, represents a substantial challenge for industrial sectors due to its deleterious effects on both human health and ecological systems [1]. This scenario reinforces the imperative for environmental preservation and the advancement of sustainable practices. In this context, the development of cost-effective and efficient alternatives for the treatment of industrial effluents is essential to mitigate the risk of environmental contamination and to prevent the undue accumulation of hazardous substances [2]. The improper disposal of toxic metals into soil or aquatic environments can lead to their accumulation in biological systems, resulting in ecosystem contamination. These metals may subsequently be transferred across trophic levels, posing risks to a wide range of organisms [3]. Cadmium (Cd) and lead (Pb) are toxic elements that can cause a range of adverse effects on human and animal health, even at low concentrations [4]. Although zinc (Zn) is an essential trace element, it can become toxic when present at elevated levels [5]. Compounds of these metals are widely used in various industrial sectors, particularly in steel manufacturing, plastics, fuels, and agricultural chemicals [6]. According to the World Health Organization [7], the maximum permissible concentrations in potable water are 0.003 mg L^{-1} for Cd, 0.01 mg L^{-1} for Pb, and 3–5 mg L^{-1} for Zn.

Conventional methods for the removal of heavy metals from wastewater predominantly rely on chemical and physical processes. These techniques are widely employed due to their proven efficacy in reducing concentrations of toxic metals. Among the most commonly used approaches are cementation, chemical precipitation, ion exchange, ion flotation, adsorption, and membrane filtration [8]. However, recent studies have demonstrated the potential of bioremoval technologies, particularly those employing microbial biomass, as a more cost-effective alternative to conventional treatment methods [9]. In particular,

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bacterial biomass can interact with metallic compounds through various mechanisms, including intracellular bioaccumulation, surface adsorption on the cell wall, metal reduction, volatilization, bioprecipitation, and biomineralization. These processes often involve the action of organic molecules such as enzymes, exopolysaccharides (EPS), metallothioneins, phosphates, and organic acids [10,11].

S. marcescens is a Gram-negative bacterium belonging to the order Enterobacterales and the family Yersiniaceae. S. marcescens CCMA1010 was isolated from coffee processing wastewater and deposited in the Culture Collection of Agricultural Microbiology (CCMA) at the Department of Biology, Federal University of Lavras (UFLA), Brazil [12]. In a previous study, [13] identified the chromosomal gene zntR (NCBI accession number: MH844628) in this strain, this gene encodes a regulatory protein that controls the expression of zntA, thereby inducing the transcription of the ZntA efflux pump. The ZntA protein mediates the export of divalent metal ions such as Zn^{2+}, Pb^{2+}, and Cd^{2+} out of the bacterial cell, enabling the microorganism to tolerate and survive in metal-contaminated environments [14,15]. Numerous studies have reported that various S. marcescens strains possess bioremediation potential, including the biodegradation of hydrocarbons and antibiotics [16,17]. Additionally, these strains have demonstrated the capacity for the bioremoval of heavy metals such as Pb^{2+}, Zn^{2+}, Cd^{2+}, Cr^{6+}, and Ni^{2+} [18–20].

The bioremoval of heavy metals using bacterial biomass is a powerful and cost-effective tool for environmental decontamination, offering eco-friendly and highly efficient solutions. However, several factors can influence this process, including pH, metal and biomass concentrations, contact time, and temperature [21]. The selection of optimal parameters using RCCD is important to identify the statistical variables that show significance in the models, with the aim of reducing experimental variability and minimizing biological effects. Therefore, this study aimed to evaluate the impact of multi-metal contamination (Cd^{2+}, Pb^{2+}, and Zn^{2+}) on the growth of S. marcescens CCMA1010 and to optimize the bioremoval process of these metals using the Rotatable Central Composite Design (RCCD). The study seeks to identify optimal conditions for bioremoval and enhance the understanding of the effects of multi-metal contamination on the bioremoval process.

2. Materials and methods

2.1. Bacterial culture

The S. marcescens CCMA1010 strain, which is part of the Culture Collection of Agrarian Microbiology at the Federal University of Lavras (CCMA/UFLA), was reactivated in nutrient broth (NB) medium (5 % peptone and 3 % yeast extract) under shaking conditions at 150 rpm and 28 °C until reaching a concentration of approximately 9 log CFU/mL.

2.2. Minimum inhibitory concentration (MIC)

The resistance of the metals was assessed by determining the minimum inhibitory concentration (MIC) in LB culture medium (1 % tryptone, 0.5 % yeast extract, 1 % NaCl, and 15 % agar), supplemented with lead nitrate, cadmium nitrate, and zinc nitrate (Sigma-Aldrich, St. Louis, MO, USA). Initially, the strain was reactivated on a plate containing solid LB medium without metals at 28°C for 24 hours. Subsequently, the biomass was plated onto solid LB medium supplemented with varying concentrations of each metal (0, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 mM). Bacterial growth was evaluated after 24 hours and observed for up to 7 days. Positive controls were performed by growing the bacteria in the culture medium without metals. All experiments were conducted in triplicate. The MIC was defined as the lowest metal concentration that completely inhibited bacterial growth.

2.3. Effect of metals in bacterial growth

The MIC values determined the conditions for the bacterial growth curve in liquid LB culture medium supplemented with metals. The S. marcescens CCMA1010 strain was standardized to an optical density (OD) of 0.6 at 600 nm using a spectrophotometer. An aliquot of 0.6 mL was inoculated into 300 mL of LB broth supplemented with metals in concentration 5 mM, 4 mM and 0.1 mM for Zn^{2+}, Pb^{2+} and Cd^{2+}, respectively. For the control treatment, LB broth without metals was used. Bacterial growth was monitored every 3 hours over a 34-hour period using a spectrophotometer.

2.4. Experimental conditions for metals bioremoval

S. marcescens CCMA1010 was cultivated in LB culture medium until the log phase. An aliquot of 10 mL was centrifuged at 4 °C at 7155.2 × *g* for 10 min. The bacterial biomass was resuspended in 1 mL of 0.1 % saline solution. The experiment was conducted in 50 mL Falcon tubes, each containing 9 mL of Pb^{2+}, Cd^{2+}, and Zn^{2+} solutions, with concentration determined by RCCD (Table 1). A total of 27 runs of the RCCD were applied to assess four variables: pH, Cd^{2+}, Pb^{2+}, and Zn^{2+}. The range of the variables used in the experimental design is presented in Table 1. The experiment was conducted using three central points along with minimum (−α) and maximum (+α) axial points, totaling 27 runs, designed using the Stat-Ease 360 software.

Flame atomic absorption spectrometry using a novAA 350 instrument (Analytik Jena) was employed to quantify the metal concentrations. The bioremoval of metals was calculated using equation (1):

$$q = \frac{(Co - Ce).V}{M} \quad (1)$$

In this equation, q is the amount of metallic ion biosorbed (mg g^{-1}), C_o is the initial concentration of the metal (mg L^{-1}), C_e is the final concentration of the metal (mg L^{-1}), V is the volume of the solution (L), and M is the mass of the biosorbent (g).

2.5. Fourier transform infrared spectrometry (FTIR)

FTIR was employed to evaluate the interactions between metallic ions and the cell surface of *S. marcescens* CCMA1010. Bacterial samples were exposed to metals in 150 mL flasks containing 90 mL of distilled water supplemented with Pb^{2+}, Cd^{2+}, and Zn^{2+} at concentrations of 590 mg L^{-1}, 470 mg L^{-1}, and 60 mg L^{-1}, respectively. The values were determined based on the RCCD analysis. Additionally, a treatment with Pb^{2+} alone (lead treatment) was included to assess the potential for phosphate synthesis under lead exposure. Microbial biomass was inoculated at a proportion of 10 % (v/v). The control treatment consisted of bacterial biomass not exposed to metals. After exposure, the biomass was centrifuged at 7155.2 × *g* and dried in a forced-air incubator at 60°C. The dried biomass was then mixed with KBr at a proportion of 1 % (v/v) for FTIR analysis. Spectra were acquired in the range of 400–4000 cm^{-1}, with 32 scans and a resolution of 4 cm^{-1}, using an FTS 3000 Excalibur Digilab spectrometer [22].

Table 1
Central Composite Design analysis for bioremoval of Cd^{2+}, Pb^{2+}, and Zn^{2+}.

Independent variable		Range				
		−1.41	−1	0	+1	+1.41
pH	A	2	3	4	5	6
Cd^{2+} (mg L^{-1})	B	110	230	350	470	590
Pb^{2+} (mg L^{-1})	C	80	250	420	590	750
Zn^{2+} (mg L^{-1})	D	9	25	40	60	75

3. Results

3.1. Minimal inhibitory concentration (MIC)

The resistance of S. marcescens CCMA1010 to heavy metals was evaluated by determining the minimum inhibitory concentration (MIC), using 6 mM as the highest concentration tested. The strain exhibited differential tolerance to the metals, with resistance decreasing in the order Zn^{2+}>Pb^{2+}>Cd^{2+}, corresponding to MIC values of 5 mM, 4 mM, and 0.1 mM, respectively.

3.2. Effect of cd^{+2}, pb^{+2} and zn^{+2} in bacterial growth

The MIC values were used to establish the growth dynamics of S. marcescens CCMA1010. Bacterial growth was monitored for up to 34 hours in the presence of Cd^{2+}, Pb^{2+}, and Zn^{2+} individually, as well as in a multi-contaminated medium, and compared to the control treatment (without metals) (Fig. 1). It was observed that the control treatment exhibited the highest bacterial growth, reaching the stationary phase at approximately 24 hours. Treatments supplemented with Zn^{2+}, Cd^{2+}, and the multi-contaminated showed an extended lag phase of up to approximately 10 hours. In contrast, the medium supplemented with Pb^{2+} presented a lag phase similar to the control (Fig. 1).

3.3. Optimization of Cd^{2+}, Pb^{2+} and Zn^{2+} bioremoval

The optimization of Cd^{2+}, Pb^{2+}, and Zn^{2+} bioremoval was conducted using a Rotatable Central Composite Design (RCCD), which enabled the evaluation of the interactive effects among the variables pH, and metal concentrations on the biosorption process. A total of 27 experimental runs were performed, including factorial points, axial points (±α), and three central points, allowing the construction of a predictive model and identification of optimal conditions for maximal metal bioremoval. The results were analyzed using the Stat-Ease 360 software, which generated the models and diagnostic plots for process interpretation.

The statistical models for Cd^{2+}, Pb^{2+} and Zn^{2+}were showing the analysis of variance (ANOVA), with detailed parameter values available in the Supplementary Material (SM) (Table 1-SM, Table 2-SM and Table 3-SM for Cd^{2+}, Pb^{2+} and Zn^{2+} uptake (q), respectively). For Cd^{2+} (q), the model F-value of 30.22 indicates that the model is statistically significant, with only a 0.01 % probability that such a high F-value could result from random noise. P-values lower than 0.0500 indicate significant model terms, in this case, the terms B, B^2, and D^2 are significant. However, the Lack of Fit F-value of 93.27 suggests a significant lack of fit, with only a 1.07 % probability that such a high value could be attributed to noise. For Pb^{2+} (q), the model F-value of 4.86 also demonstrates statistical significance, with a 0.46 % likelihood of occurring by chance. In this case, the terms C and C^2 were identified as significant. The Lack of Fit F-value of 14.74 indicates that the lack of fit is not significant when compared to the pure error, with a 6.52 % probability of this result being due to random variation. For Zn^{2+} (q), the model F-value

Table 2
Experimental design of RCCD for variables (Cd^{2+}; Pb^{2+}, Zn^{2+} and pH) and Response for Bioremoval (q) for Cd^{2+}, Pb^{2+} and Zn^{2+}.

Run order	Variables				Response (q)		
	pH	mg L^{-1}			mg g^{-1}		
		Cd	Pb	Zn	Cd	Pb	Zn
1	1	−1	1	1	0	243.3	17
2	1.41	0	0	0	18.9	34.3	0
3	1	1	1	1	157.1	188.8	18.7
4	1	−1	−1	−1	0	0	0
5	−1	−1	−1	−1	0	0	0
6	0	0	0	0	20	64.4	0
7	0	0	−1.41	0	20.3	338.9	0
8	1	−1	−1	1	0	0	19.2
9	0	0	1.41	0	14.5	372	0
10	−1	1	1	−1	158.3	185.8	0
11	1	1	−1	−1	154.5	0	25.5
12	1	−1	1	−1	0	212.6	27.2
13	0	0	0	0	24.8	26.7	0
14	−1	−1	1	−1	0	260.5	0
15	−1.41	0	0	0	27.1	16.8	0
16	−1	−1	−1	1	0	0	19.7
17	0	0	0	−1.41	39.8	42.2	0
18	−1	1	1	1	163.1	208.3	20.4
19	−1	1	−1	1	162.3	0	20.1
20	0	0	0	1.41	112.2	177.7	45.2
21	0	0	0	0	22.4	45.6	0
22	1	1	1	−1	164.3	212.5	0
23	1	1	−1	1	223.9	11.7	24.9
24	0	−1.41	0	0	0	26	0
25	0	1.41	0	0	298.8	31.5	0
26	−1	−1	1	1	0	185	0
27	−1	1	−1	−1	153.9	0	0

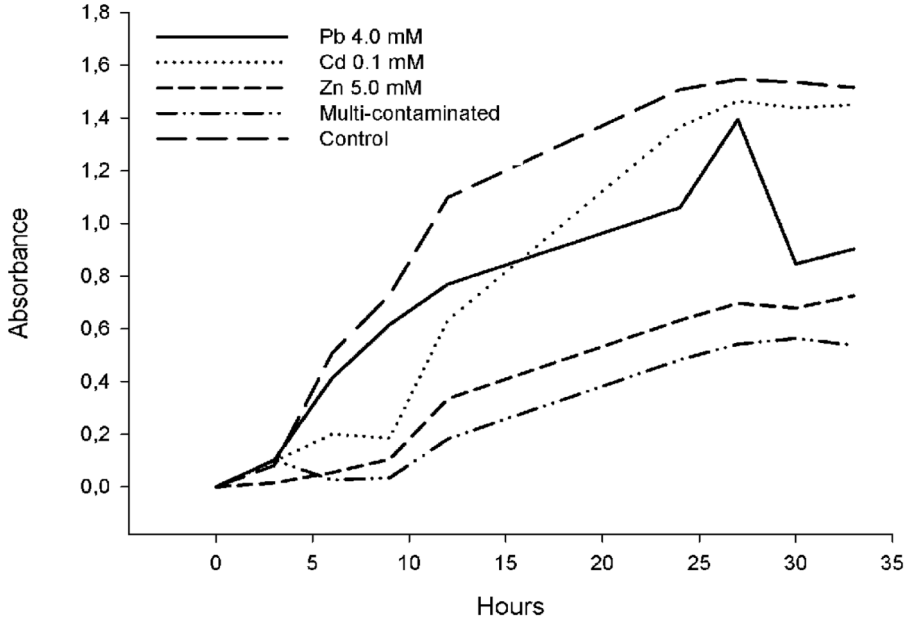

Fig. 1. *S. marcescens* CCMA1010 growth in presence of Pb^{2+}, Cd^{2+}, Zn^{2+} individually, multi-contaminated and control treatment (without metals).

of 2.21 suggests that the model is not statistically significant, with an 8.79 % chance of such a value occurring due to noise. Accordingly, the variable D is considered a non-significant model term based on its P-value.

In Fig. 2 (a, b, and c), the residuals observed for Cd^{2+} (q) are satisfactory, with no apparent outliers. Fig. 2(d) shows the predicted values plotted against the experimentally observed values, indicating good agreement. However, in Fig. 3 (a, b, and c), the residuals for Pb^{2+} (q) reveal an outlier in run 21 ($q = 338.90$), corresponding to the axial point -1.41 for the initial Pb^{2+} concentration (mg L^{-1}). This outlier significantly affects the model's performance and contributes to the lower adjusted R^2 observed for Pb^{2+} bioremoval (67 %). Fig. 3(d) show the predicted values plotted to Pb^{2+} uptake.

The fit summary for the quadratic models is presented in Table 4 - SM, outlining the results for Cd^{2+} and Pb^{2+} bioremoval (q). Both responses were best described by a quadratic model (P-value < 0.05). The coefficient of determination (R^2) was 94 % for Cd^{2+} (q), indicating a strong correlation between the observed and predicted values. For Pb^{2+} (q), the R^2 value was 67 %, suggesting a moderate predictive capacity.

The significance of model terms, shown in Table 5-SM, reveals that only the initial concentrations of Cd^{2+} and Pb^{2+} significantly influenced their respective bioremoval responses (P-value < 0.05).

No significant effects were observed for pH or for the presence of multiple metals in the solution (P-value > 0.05). These findings suggest that neither pH nor metal–metal interactions resulted in a quadratic response for Cd^{2+} and Pb^{2+} uptake, in the range studied. Therefore, regression equations were developed based solely on the significant predictors for Cd^{2+} and Pb^{2+} bioremoval, as presented in Eq. (2) and 3, respectively.

$$\begin{aligned} Y \;=\; & 32.14 + (+2.53 * A) + (+87.99 * B) + (-3.00 * C) + (+8.88 * D) \\ & + (+3.88 * AB) + (-3.88 * AC) + (+3.06 * AD) + (-3.23 * BC) \\ & + (+4.71 * BD) + (-5.01 * CD) + \left(-8.46 * A^2\right) + \left(+54.73 * B^2\right) \\ & + \left(-11.26 * C^2\right) + \left(+18.03 * D^2\right) \end{aligned} \tag{2}$$

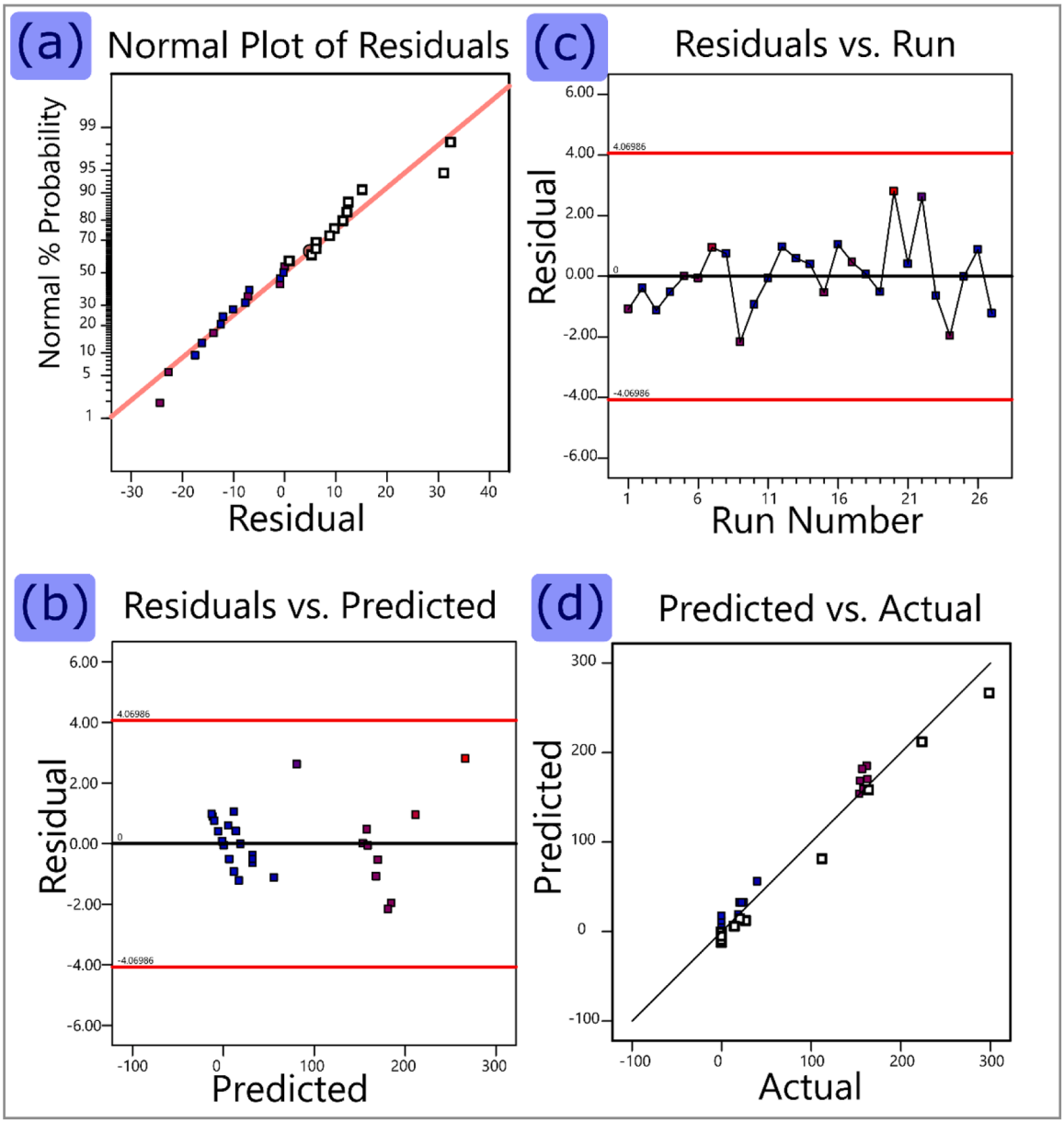

Fig. 2. Residual graphs for Cd^{2+} bioremoval (q) model. (a) Normal Plot of Residuals, (b) Residuals vs. Predicted, (c) Residual vc. Run and (d) Predicted vs. Actual.

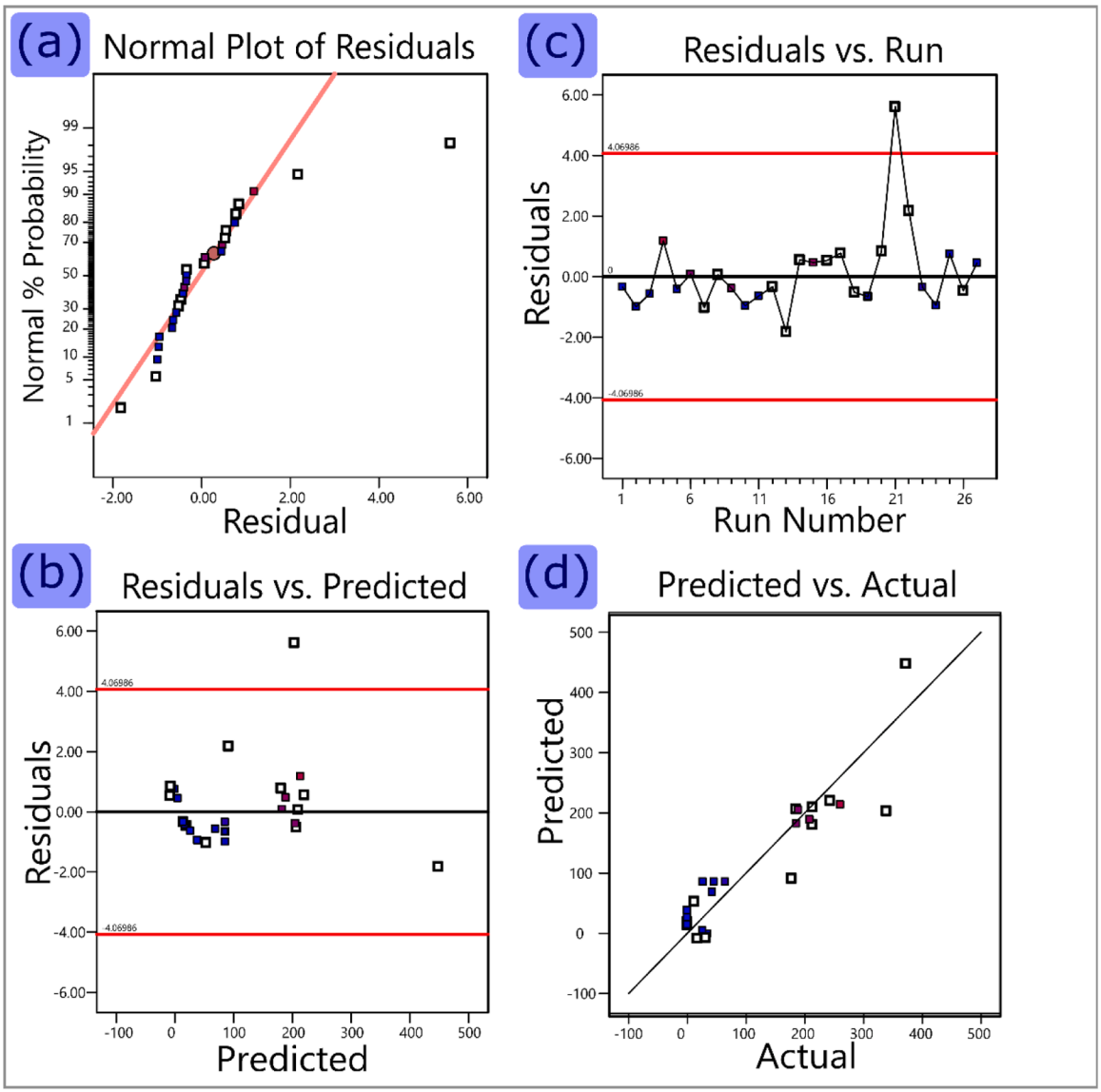

Fig. 3. Residual graphs for Pb^{2+} bioremoval (q) model. (a) Normal Plot of Residuals, (b) Residuals vs. Predicted, (c) Residual vc. Run and (d) Predicted vs. Actual.

$$Y = 85.56 + (+2.70 * A) + (-4.32 * B) + (+86.59 * C) + (+7.86 * D) + (+0.53 * AB) + (+0.36 * AC) + (+4.48 * AD) + (-7.35 * BC) + (+3.45 * BD) + (-3.60 * CD) + (-45.00 * A^2) + (-43.40 * B^2) + (+119.94 * C^2) + (-2.80 * D^2) \quad (3)$$

In Eq. (2) and 3, Y represents the predicted bioremoval of Cd^{2+} and Pb^{2+} (mg g^{-1}), respectively. The variables A, B, C, and D correspond to pH, initial Cd^{2+} concentration, initial Pb^{2+} concentration, and initial Zn^{2+} concentration, respectively.(Fig. 4 and Fig. 5)

3.4. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectral analysis of S. marcescens CCMA1010 indicates the presence of various functional groups (Fig. 6). The broad peak around 3500 cm^{-1} is typically associated with -OH stretching vibrations, suggesting the presence of alcohol or phenol groups. The region near 1500 cm^{-1} corresponds to the -COOH stretching vibration, indicative of carboxylic acids. The peak at 1539 cm^{-1} is attributed to CH_2 (methylene) groups, while the peak at 1654 cm^{-1} corresponds to CH_3 (methyl) group vibrations, reflecting the presence of alkyl chains. Peaks at 544, 583, and 1003 cm^{-1} are linked to the asymmetric angular vibrations of PO_4^{3-} groups, indicating phosphate content. The band around 1230 cm^{-1} is associated with SO_3^- groups, characteristic of sulfonates, and the peak near 800 cm^{-1} is assigned to S–O stretching in the C–SO_3^- group, confirming the presence of sulfonated compounds.

4. Discussion

Microorganisms isolated from contaminated environments have demonstrated potential for use in bioremediation processes due to their resistance mechanisms against metal-induced stress [23]. *S. marcescens* CCMA1010 was isolated of wastewater coffee processing, that has 130 mg L^{-1} of cadmium [12]. The elevated metal concentrations commonly found in agricultural waste are often associated with the use of inorganic agricultural inputs rich in heavy metals [24,25]. *S. marcescens* strains from diverse origins have been studied and have demonstrated potential for the bioremediation of nickel, cadmium, lead, chromium, and zinc. This potential is attributed to mechanisms such as metal reduction, siderophore production, metallothionein expression, and biosorption on the cell wall surface [18,19,26–28]. Different microorganisms may possess genes that encode specific proteins and enzymes involved in heavy metal detoxification [29], such as czc, zntA, cadA, and pbrA in *Cupriavidus metallidurans* these resistance genes encode transmembrane

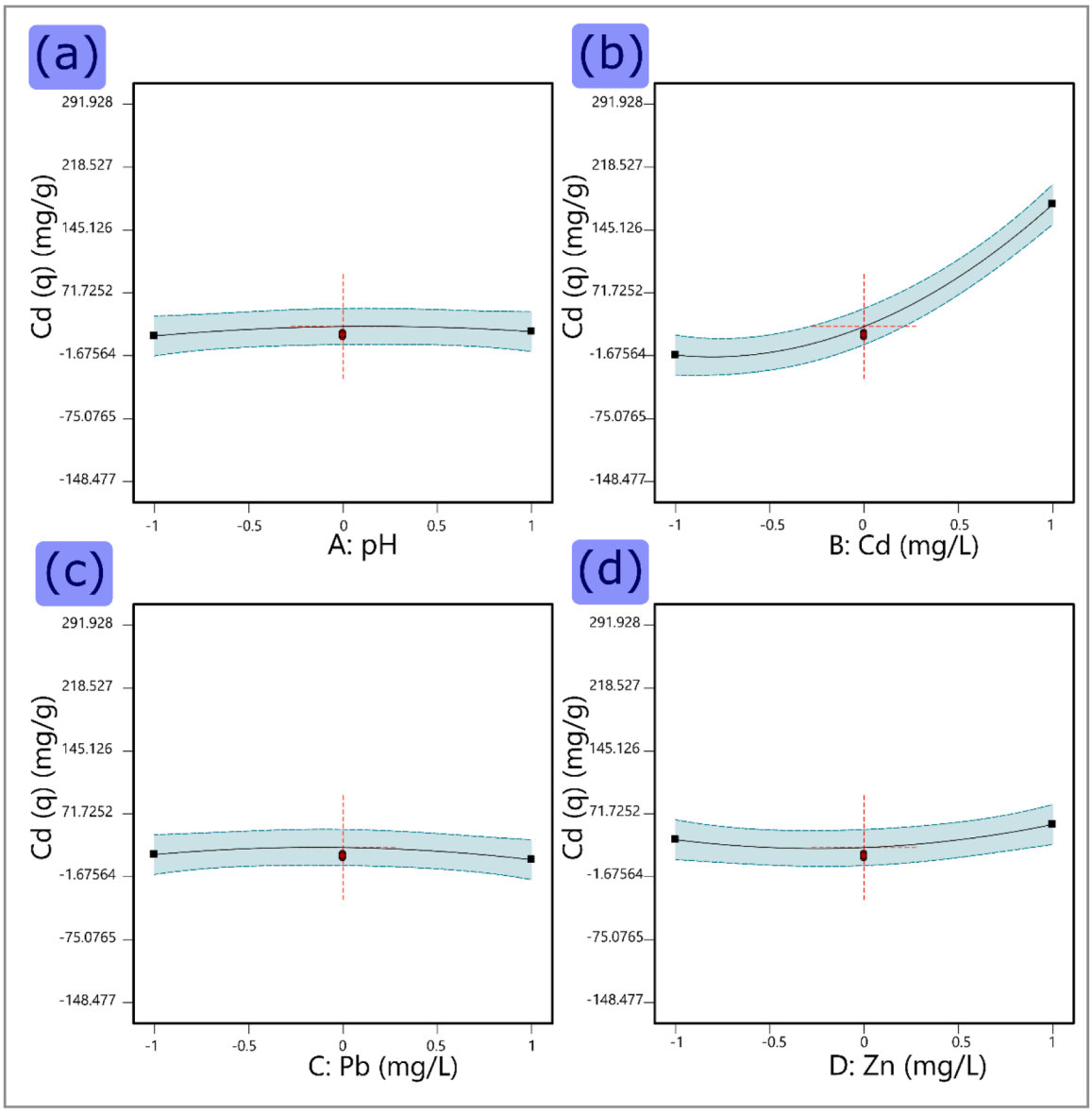

Fig. 4. Interaction effects of parameters in Cd^{2+} biossorption.

efflux pumps, enabling the efficient expulsion of Zn^{2+}, Cd^{2+}, Pb^{2+}, Co^{2+}, and Ni^{2+} under metal stress [30]. *C. metallidurans* CH34 utilizes the pbr operon to confer Pb^{2+} resistance through mechanisms such as transport, efflux, sequestration, biomineralization, and precipitation. This operon includes gene homologs such as pbrR, pbrR2, and pbrD, which are involved in Pb^{2+} binding and detoxification [31].

Microbial-induced phosphate precipitation is an important mechanism for reducing the toxicity and bioavailability of heavy metals [32, 33]. In the case of lead, precipitation as lead phosphate is an extracellular resistance strategy, often evidenced by a brown coloration of colonies or the surrounding culture medium [34], as observed in this study (Fig. 1- SM). Providencia alcalifaciens 2EA exhibited a characteristic brown color, indicative of lead bio-precipitation, converting soluble lead into insoluble $Pb_9(PO_4)_6$, this transformation was confirmed by SEM-EDX and X-ray diffraction (XRD) analyses, and was catalyzed by microbial phosphatase activity [35]. Biomineralization involves the hydrolysis of polymeric organic phosphorus (POP), which is transported into the microbial cell and subsequently converted into inorganic phosphate (PO_4^{3-}) through enhanced metabolic activity mediated by phosphatase or phytase enzymes. The resulting PO_4^{3-} is then exported from the cell, where it binds to lead ions on the cell surface, facilitating the formation of insoluble lead-phosphate complexes [33] (Fig. 8).

Before POP hydrolysis occurs, lead (and other metals) is absorbed by the bacterial cell. The intracellular accumulation of metals leads to a decrease in zntR transcription, due to the negative feedback regulation exerted by ZntR on zntA expression [20,36]. ZntA, a metal-efflux protein, plays a key role in maintaining intracellular metal homeostasis, and its expression is regulated by the transcriptional activator ZntR [14]. When S. marcescens CCMA1010 was cultivated in medium supplemented with lead (up to 120 mg L^{-1}), an increase in zntR expression was observed. Moreover, at initial lead concentrations above 120 mg L^{-1}, after 168 hours of incubation, the active biomass removed more lead than at lower concentrations. This enhanced removal required the activity of the ZntA efflux protein. These findings suggest that ZntR, when bound to Pb^{2+}, may regulate its own expression without necessarily repressing zntA transcription. This opens up discussion about the precise regulatory role and functionality of the ZntR protein. Additionally, the mechanism of metal extrusion was supported by the observation that active biomass exhibited lower lead accumulation compared to inactive biomass [20]. This result aligns with findings by [34], who reported that live Bacillus cereus biomass exhibited lower metal bioremoval efficiency compared to dead biomass. This phenomenon is attributed to the

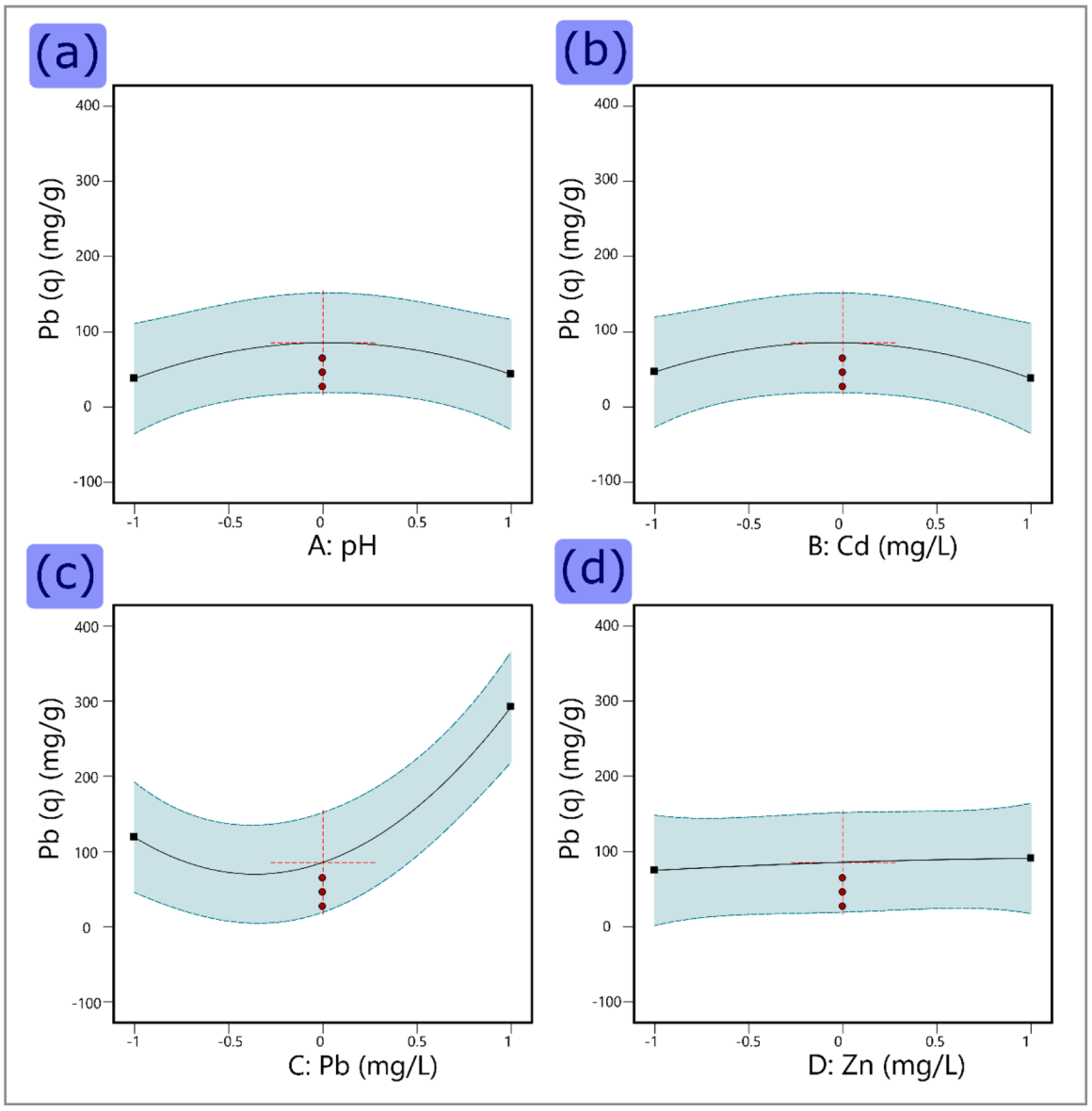

Fig. 5. Interaction effects of parameters in Pb^{2+} biossorption.

autoclaving process, which disrupts the cell wall and exposes additional functional groups that enhance metal binding. In this context, dead cells of Gram-positive bacteria tend to be more effective biosorbents than those of Gram-negative bacteria. Conversely, live Gram-negative bacteria generally demonstrate greater bioremoval efficiency than live Gram-positive bacteria, likely due to their thinner peptidoglycan layer and more complex outer membrane structure, which facilitates active metal transport and extrusion [37].

The present study suggests that biomineralization, through the synthesis of metal precipitates (potentially lead-phosphate) serves as a resistance mechanism employed by S. marcescens CCMA1010 (Fig. 7). Moreover, phosphate synthesis may be linked to enhanced biological activity, particularly under conditions of metal-induced stress [13]. This hypothesis is supported by FTIR spectral analysis (Fig. 6). When exposed to metals, S. marcescens CCMA1010 exhibited functional groups indicative of specific biochemical responses. Vibrational shifts at 544, 583, and 1003 cm^{-1} correspond to phosphate groups, which are commonly associated with the precipitation of metals as metal phosphate complexes. Additionally, lead exposure resulted in peaks at 1230 cm^{-1}, suggesting the presence of S–O linkages, and around 800 cm^{-1}, indicating the possible formation of sulfate compounds [38,39]. Lead crystallization as phosphate is a metabolically dependent mechanism. The reaction between target metals and POP is part of the bacterial defense system. Although dead bacterial cells can also mediate this interaction in a metabolically independent manner, the efficiency is lower due to the passive nature of the interaction between reactive groups on the cell surface and metal ions, when compared to live biomass [37]. *S. marcescens* CCMA1010, when inoculated in a medium supplemented with Pb^{2+}, exhibited higher metabolic activity and cell growth comparable to that in lead-free medium, indicating that its defence system is metabolically dependent [13].

Metal-induced stress by Cd^{2+} and Zn^{2+} directly affected the lag phase of S. marcescens CCMA1010. Similar observations were reported by [37] in Pseudomonas fluorescens and by [15] in Bacillus cereus, which exhibited an extended lag phase of up to 10 hours in the presence of heavy metals. However, conducting bioremoval studies in multi-metal environments can enhance the understanding of mechanisms and application potential, given the real complexity of contaminated waste [40,41]. In study with multiple metals, the adsorption of Pb^{2+} occurred efficiently even in the presence of both Cr^{4+} and Cd^{2+}. In contrast, Cd^{2+} adsorption was only slightly affected by the presence of Pb^{2+}, whereas Cr^{4+} adsorption was significantly influenced by the co-occurrence of

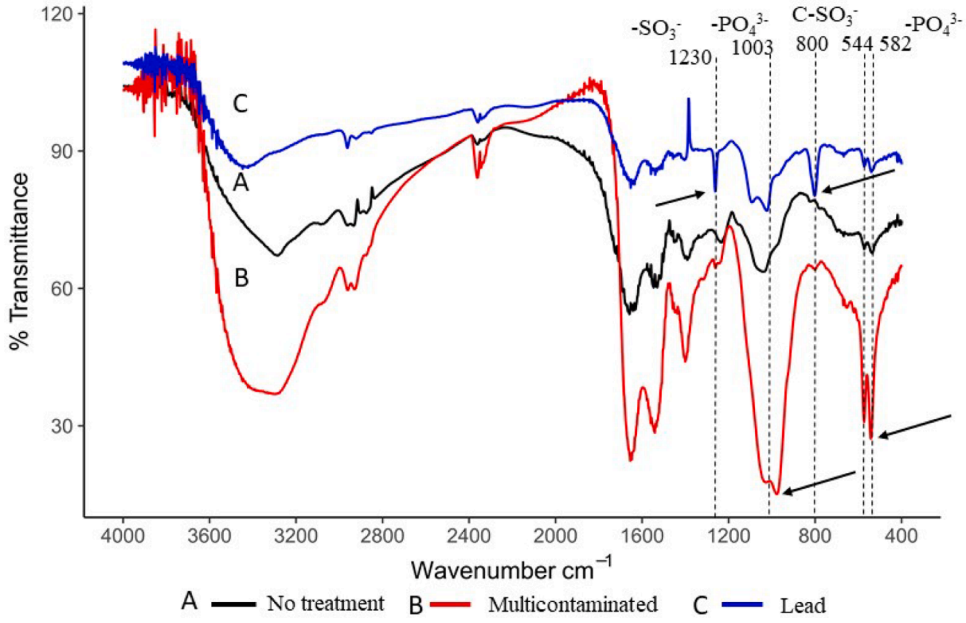

Fig. 6. FTIR analysis of *S. marcescens* CCMA1010 biomass in heavy-metals-free culture, exposed to multi-metals (Cadmium, Lead and Zinc) and exposed to Lead.

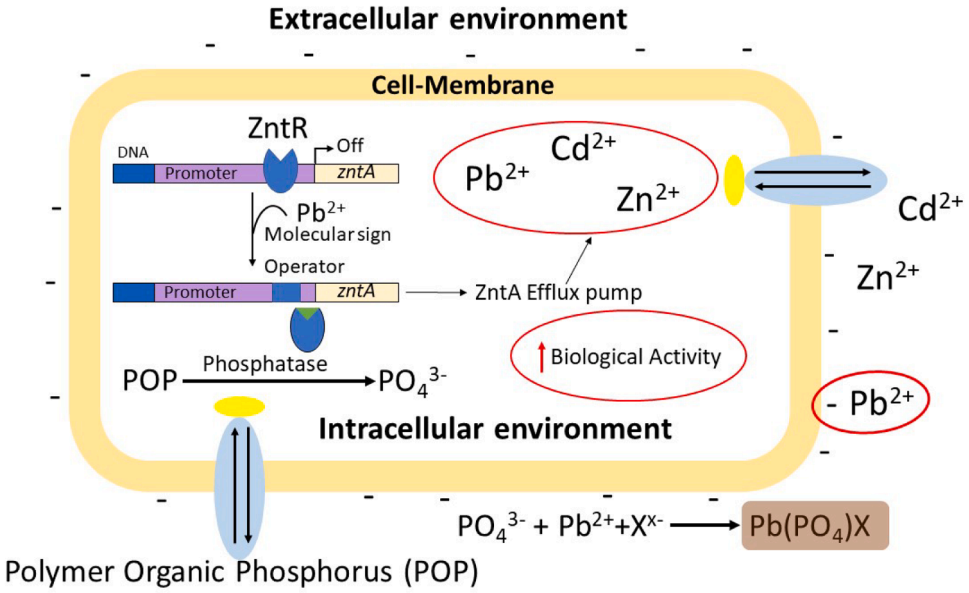

Fig. 7. Schematic diagram for probably lead bioremoval strategies *S. marcescens* CCMA1010.

both Pb^{2+} and Cd^{2+} [42]. This result is particularly important, as in the present study, bioremoval was not affected by the co-existence of multiple metals, likely due to the action of POP which play a key role in lead bioprecipitation and are not directly involved in the uptake of Zn^{2+} or Cd^{2+} [37]. Moreover, the pH range studied was not a significant factor for metal uptake. However, it is well known that pH can play a crucial role in the bioremoval process, as it influences metal precipitation and the saturation of binding sites on the bacterial cell wall [43]. *Lactobacillus plantarum* MF042018, isolated from marine sediment, demonstrated the ability to biosorb Cd^{2+} and Pb^{2+} at pH 2.0 and a low incubation temperature (22 °C) [44]. A strain of Aspergillus flavus was used to model the biosorption of both Cr and Mn at pH 10.0 [21] For Pb^{2+} bioremoval, Aeromonas hydrophila was employed in a model operating at pH 5.0 [45]. These results highlight that the optimal pH values for biosorption can vary depending on the properties of the biosorbent and the characteristics of the medium [46].

This is investigations have unveiled that *S. marcescens* CCMA1010 is a promising microorganism with significant potential for bioremediation owing to its remarkable capacity for metal biosorption. The ability to thrive in a medium supplemented with metals does not solely determine biosorption; rather, it is likely attributed to the expression of active resistance genes that facilitate efflux pumps, transporting metals outside the cells. Despite its potential in biosorption, the application in waste contaminated with multimetals necessitates further study due to the intricate interactions between multiple metals, microorganisms, and abiotic factors.

5. Conclusion

S. marcescens CCMA1010 biomass demonstrated the ability to grow in a medium containing multiple metals, with notable tolerance to Pb^{2+}, as growth was comparable to that observed in the control treatment. The

bacterial biomass produced an organic compound that induced vibrational changes in phosphate and sulphate functional groups, which may have contributed to Pb^{2+} bioprecipitation, resulting in visible colour changes in both the bacterial colonies and the culture medium. Although the Pb^{2+} uptake model was statistically significant in this study, the specific biochemical mechanism involved has not yet been fully clarified, and further research is required to confirm this proposed pathway. The uptake model for Cd^{2+} was also significant, but the specific mechanism could not be determined. In contrast, the model for Zn^{2+} uptake was not statistically significant. Additionally, pH did not significantly influence metal uptake under the conditions studied. The ANOVA results showed no statistically significant interaction effects among multiple metals in the bioremoval process.

Ethical approval and consent for to participate

Not applicable.

Data availability

Data available within the article.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Gustavo Magno dos Reis Ferreira: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Rosane Freitas Schwan:** Writing – review & editing, Validation, Supervision, Conceptualization. **Cristina Ferreira Silva:** Writing – review & editing, Writing – original draft, Validation, Supervision, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nexres.2025.101136.

References

[1] N.K. Soliman, A.F. Moustafa, Industrial solid waste for heavy metals adsorption features and challenges; a review, J. Mater. Res. Technol. 9 (2020) 10235–10253.
[2] A.K. Priya, M. Muruganandam, A. Kumar, N. Senthilkumar, M. Shkir, B. Pandit, M. Imran, C. Prakash, M. Ubaidullah, Recent advances in microbial-assisted degradation and remediation of xenobiotic contaminants; challenges and future prospects, J. Water Process Eng. (2024) 60.
[3] N.B. Saidon, R. Szabó, P. Budai, J. Lehel, Trophic transfer and biomagnification potential of environmental contaminants (heavy metals) in aquatic ecosystems, Environ. Pollut. (2024) 340.
[4] S. Satarug, G.C. Gobe, D.A. Vesey, K.R. Phelps, Cadmium and lead exposure, nephrotoxicity, and mortality, Toxics 8 (2020) 1–42.
[5] C.T. Chasapis, P.S.A. Ntoupa, C.A. Spiliopoulou, M.E. Stefanidou, Recent aspects of the effects of zinc on human health, Arch Toxicol 94 (2020) 1443–1460.
[6] H. Çelebi, G. Gök, O. Gök, Adsorption capability of brewed tea waste in waters containing toxic lead(II), cadmium (II), nickel (II), and zinc(II) heavy metal ions, Sci Rep 10 (2020), https://doi.org/10.1038/s41598-020-74553-4.
[7] WHO, 2017. Guidelines for drinking-water quality FOURTH EDITION INCORPORATING THE FIRST ADDENDUM.
[8] S. Krishnan, N.S. Zulkapli, H. Kamyab, S.M. Taib, M.F.B.M. Din, Z.A. Majid, S. Chaiprapat, I. Kenzo, Y. Ichikawa, M. Nasrullah, S. Chelliapan, N. Othman, Current technologies for recovery of metals from industrial wastes: an overview, Env. Technol Innov (2021) 22.
[9] R. Shrestha, S. Ban, S. Devkota, S. Sharma, R. Joshi, A.P. Tiwari, H.Y. Kim, M. K. Joshi, Technological trends in heavy metals removal from industrial wastewater: a review, J Env. Chem Eng (2021) 9.
[10] Gouda S.A., Taha A., 2023. Biosorption of heavy metals as a new alternative method for wastewater treatment: a review.
[11] J. Chen, Y. Tian, Hexavalent chromium reducing bacteria: mechanism of reduction and characteristics, Environ. Sci. Pollut. Res. (2021), https://doi.org/10.1007/s11356-021-13325-7/Published.
[12] J.F. Pires, L. Cardoso, S. de, R.F. Schwan, C.F. Silva, Diversity of microbiota found in coffee processing wastewater treatment plant, World J Microbiol Biotechnol 33 (2017), https://doi.org/10.1007/s11274-017-2372-9.
[13] GM dos R Ferreira, J.F. Pires, L.S. Ribeiro, J.D. Carlier, M.C. Costa, R.F. Schwan, C. F. Silva, Impact of lead (Pb2+) on the growth and biological activity of Serratia marcescens selected for wastewater treatment and identification of its zntR gene—A metal efflux regulator, World J Microbiol Biotechnol 39 (2023), https://doi.org/10.1007/s11274-023-03535-1.
[14] V. Schulz, C. Schmidt-Vogler, P. Strohmeyer, S. Weber, D. Kleemann, D.H. Nies, M. Herzberg, Behind the shield of Czc: zntR controls expression of the gene for the zinc-exporting P-type ATPase ZntA in cupriavidus metallidurans, J Bacteriol 203 (2021), https://doi.org/10.1128/JB.00052-21.
[15] F. Huang, C.L. Guo, G.N. Lu, X.Y. Yi, L.D. Zhu, Z. Dang, Bioaccumulation characterization of cadmium by growing Bacillus cereus RC-1 and its mechanism, Chemosphere 109 (2014) 134–142, https://doi.org/10.1016/j.chemosphere.2014.01.066.
[16] Y. Huang, H. Zhou, G. Zheng, Y. Li, Q. Xie, S. You, C. Zhang, Isolation and characterization of biosurfactant-producing Serratia marcescens ZCF25 from oil sludge and application to bioremediation, Environ. Sci. Pollut. Res. 27 (2020) 27762–27772, https://doi.org/10.1007/s11356-020-09006-6.
[17] P. Bhatt, C.H. Jeon, W. Kim, Tetracycline bioremediation using the novel Serratia marcescens strain WW1 isolated from a wastewater treatment plant, Chemosphere 298 (2022), https://doi.org/10.1016/j.chemosphere.2022.134344.
[18] M. Cristani, C. Naccari, A. Nostro, A. Pizzimenti, D. Trombetta, F. Pizzimenti, Possible use of Serratia marcescens in toxic metal biosorption (removal), Environ. Sci. Pollut. Res. 19 (2012) 161–168, https://doi.org/10.1007/s11356-011-0539-8.
[19] C.N. Elizabeth, M.Y. Victoria, E.E. Nkechi, B.O. Godwin, Isolation and characterization of heavy metal tolerant bacteria from Panteka stream, Kaduna, Nigeria and their potential for bioremediation, Afr J Biotechnol 16 (2017) 32–40, https://doi.org/10.5897/ajb2016.15676.
[20] J.D. Carlier, G.M. dos Reis Ferreira, R.F. Schwan, C.F. da Silva, Costa MC, Pb2+ biosorption by Serratia marcescens CCMA 1010 and its relation with zntR gene expression and ZntA efflux pump regulation, Environ. Adv. 15 (2024), https://doi.org/10.1016/j.envadv.2023.100479.
[21] F. Dejene, F. Wubishet, R.S. Suresh Kumar, A. Manilal, A. Mamo, T. Sundaram, Optimisation of aspergilus flavus-mediated biosorption process for removal of Cr (VI) and Mn (II) ions by response surface methodology approach, Indian J Microbiol (2024), https://doi.org/10.1007/s12088-024-01328-0.
[22] M. Martins, M.L. Faleiro, A.M.R. da Costa, S. Chaves, R. Tenreiro, A.P. Matos, M. C. Costa, Mechanism of uranium (VI) removal by two anaerobic bacterial communities, J Hazard Mater 184 (2010) 89–96, https://doi.org/10.1016/j.jhazmat.2010.08.009.
[23] B. Zhou, T. Zhang, F. Wang, Microbial-based heavy metal bioremediation: toxicity and eco-friendly approaches to heavy metal decontamination, Appl. Sci. (Switz.) (2023) 13.
[24] N.S. Shaban, K.A. Abdou, N.E.-H.Y. Hassan, Impact of toxic heavy metals and pesticide residues in herbal products, Beni Suef Univ J Basic Appl Sci 5 (2016) 102–106, https://doi.org/10.1016/j.bjbas.2015.10.001.
[25] A. Alengebawy, S.T. Abdelkhalek, S.R. Qureshi, M.Q. Wang, Heavy metals and pesticides toxicity in agricultural soil and plants: ecological risks and human health implications, Toxics 9 (2021) 1–34.
[26] R. Kumar, C. Acharya, S.R. Joshi, Isolation and analyses of uranium tolerant Serratia marcescens strains and their utilization for aerobic uranium U(VI) bioadsorption, J. Microbiol. 49 (2011) 568–574, https://doi.org/10.1007/s12275-011-0366-0.
[27] M.F.A. Rahman, M.Y. Shukor, Z. Suhaili, S. Mustafa, N.A. Shamaan, M.A. Syed, Reduction of Mo(VI) by the bacterium Serratia sp. strain DRY5, J Env. Biol 30 (2009) 65–72.
[28] X. Zhu, B. Lv, X. Shang, J. Wang, M. Li, X. Yu, The immobilization effects on Pb, Cd and Cu by the inoculation of organic phosphorus-degrading bacteria (OPDB)with rapeseed dregs in acidic soil, Geoderma 350 (2019) 1–10, https://doi.org/10.1016/j.geoderma.2019.04.015.
[29] A. Garg, P. Chauhan, C. Kaur, S. Perveen, P.K. Arora, S.K. Garg, V.P. Singh, A. Srivastava, Bacterial bioremediation strategies for heavy metal detoxification: a multidisciplinary approach, Environ. Sustain. (2025), https://doi.org/10.1007/s42398-025-00369-5.
[30] C. Große, J. Scherer, G. Schleuder, D.H. Nies, Interplay between two-component regulatory systems is involved in control of Cupriavidus metallidurans metal resistance genes, J Bacteriol 205 (2023), https://doi.org/10.1128/jb.00343-22.

[31] C. Hui, B. Ma, Y. Wang, X. Yang, J. Cai, Designed bacteria based on natural pbr operons for detecting and detoxifying environmental lead: a mini-review, Ecotoxicol Env. Saf 267 (2023) 115662, https://doi.org/10.1016/j.ecoenv.2023.115662.
[32] M.M. Naik, S.K. Dubey, Lead resistant bacteria: lead resistance mechanisms, their applications in lead bioremediation and biomonitoring, Ecotoxicol Env. Saf 98 (2013) 1–7.
[33] B. Shan, R. Hao, H. Xu, J. Li, Y. Li, X. Xu, J. Zhang, A review on mechanism of biomineralization using microbial-induced precipitation for immobilizing lead ions, Environ. Sci. Pollut. Res. (2021) 30486–30498, https://doi.org/10.1007/s11356-021-14045-8/Published.
[34] U. Utami, L. Harianie, N.R. Dunyana, Romaidi, Lead-resistant bacteria isolated from oil wastewater sample for bioremediation of lead, Water Sci. Technol. 81 (2020) 2244–2249, https://doi.org/10.2166/wst.2020.281.
[35] M.M. Naik, D. Khanolkar, S.K. Dubey, Lead-resistant Providencia alcalifaciens strain 2EA bioprecipitates Pb+2 as lead phosphate, Lett Appl Microbiol 56 (2013) 99–104, https://doi.org/10.1111/lam.12026.
[36] L.M. Sheehan, J.A. Budnick, R.M. Roop, C.C. Caswell, Coordinated zinc homeostasis is essential for the wild-type virulence of Brucella abortus, J Bacteriol 197 (2015) 1582–1591, https://doi.org/10.1128/JB.02543-14.
[37] P.R. Sreedevi, K. Suresh, G. Jiang, Bacterial bioremediation of heavy metals in wastewater: a review of processes and applications, J. Water Process Eng. (2022) 48.
[38] K.L. Alvarado-Campo, M. Quintero, B. Cuadrado-Cano, M. Montoya-Giraldo, E. L. Otero-Tejada, L. Blandón, O. Sánchez, A. Zuleta-Correa, J. Gómez-León, Heavy metal tolerance of microorganisms isolated from coastal marine sediments and their lead removal potential, Microorganisms 11 (2023), https://doi.org/10.3390/microorganisms11112708.
[39] S.E. Oh, S.H.A. Hassan, J.H. Joo, Biosorption of heavy metals by lyophilized cells of Pseudomonas stutzeri, World J Microbiol Biotechnol 25 (2009) 1771–1778, https://doi.org/10.1007/s11274-009-0075-6.
[40] M. Gavrilescu, Removal of heavy metals from the environment by biosorption, Eng Life Sci 4 (2004) 219–232.
[41] M. Priyadarshanee, S. Das, Biosorption and removal of toxic heavy metals by metal tolerating bacteria for bioremediation of metal contamination: a comprehensive review, J Env. Chem Eng 9 (2021), https://doi.org/10.1016/j.jece.2020.104686.
[42] M. Priyadarshanee, S. Das, Multifaceted response surface methodology unravels competitive heavy metal adsorption affinity of immobilized biosorbent formulated from bacterial extracellular polymer of Pseudomonas aeruginosa OMCS-1, Chemosphere 368 (2024), https://doi.org/10.1016/j.chemosphere.2024.143681.
[43] M.I. Abo-Alkasem, N.H. Hassan, M.M. Abo Elsoud, Microbial bioremediation as a tool for the removal of heavy metals, Bull Natl Res Cent 47 (2023), https://doi.org/10.1186/s42269-023-01006-z.
[44] F.A. Ameen, A.M. Hamdan, M.Y. El-Naggar, Assessment of the heavy metal bioremediation efficiency of the novel marine lactic acid bacterium, Lactobacillus plantarum MF042018, Sci Rep 10 (2020), https://doi.org/10.1038/s41598-019-57210-3.
[45] S.H. Hasan, P. Srivastava, M. Talat, Biosorption of Pb(II) from water using biomass of Aeromonas hydrophila: central composite design for optimization of process variables, J Hazard Mater 168 (2009) 1155–1162, https://doi.org/10.1016/j.jhazmat.2009.02.142.
[46] M. Aryal, M. Liakopoulou-Kyriakides, Bioremoval of heavy metals by bacterial biomass, Env. Monit Assess 187 (2015), https://doi.org/10.1007/s10661-014-4173-z.