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Ureolytic *Lysinibacillus* sp. Exhibiting Calcium Carbonate Precipitation with Effective Lead (Pb) Removal

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Abstract

Industrial effluents contaminated with heavy metals are a major environmental issue, prompting the need for sustainable bioremediation methods. Microbially induced calcium carbonate precipitation (MICP) is a remediation method that offers a long-term solution for enhancing soil mechanical properties, as well as reducing pollution from heavy metals. The objectives of the current study include screening and identifying native ureolytic bacteria from soil in Karad, as well as assessing calcite precipitation by ureolytic metallotolerant bacteria. The effectiveness of the isolated bacteria in removing lead as a heavy metal was tested in the medium. This research focuses on the removal of Pb heavy metal by using the MICP method. Samples of wastewater were taken from calcareous soils and effluents contaminated with heavy metals. Ureolytic bacteria were identified using urea agar medium, and nine positive isolates were obtained by using Christensen's media. Ureolytic isolates were then screened for their tolerance to metal Pb^{2+} as well as calcium precipitation. The maximum tolerance ranged from 2 mM to 8 mM, depending on the metal ion. The potential isolate was identified through 16S rRNA gene sequencing. *Lysinibacillus fusiformis* was recognized as a urease-producing, metallotolerant bacterium with calcium precipitating. By using *Lysinibacillus fusiformis*, 85% of the lead was removed. This is to evaluate *Lysinibacillus*-mediated MICP for Pb bioremediation from under environmentally relevant conditions, treated for real wastewater applications."

Keywords: urease; bioremediation; heavy metals; microbially induced calcium precipitation [MICP]

Introduction:

Heavy metal contamination is a major global environmental concern due to its persistence, non-biodegradability, and toxicity. Among heavy metals, lead (Pb) is particularly hazardous because of its ability to bioaccumulate and biomagnify through food chains. Industrial activities such as mining, smelting, electroplating, battery manufacturing, and improper waste disposal significantly contribute to elevated Pb levels in soil and water systems (Ali, Khan and Ilahi, 2019). Chronic exposure to lead can result in neurological impairment, renal dysfunction, hematological disorders, and developmental abnormalities in humans and wildlife (Abd Elnabi et al., 2023).

Conventional physicochemical methods for lead removal, including chemical precipitation, ion exchange, membrane filtration, and adsorption, are often expensive and may generate secondary pollutants. Therefore, environmentally sustainable and cost-effective biological approaches have gained increasing attention. Microbial remediation strategies utilize natural metabolic processes to immobilize or transform heavy metals into less toxic forms(Jalilvand et al., 2020).

Microbially induced calcium carbonate precipitation (MICP) is a promising biomineralization process in which urease-producing bacteria hydrolyze urea to generate carbonate ions and ammonia, resulting in an increase in pH and subsequent precipitation of calcium carbonate ($CaCO_3$). (Hammes et al., 2003).The formed carbonate minerals can immobilize heavy metals through co-precipitation,

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adsorption, and structural incorporation within calcite matrices. Recent studies have demonstrated the applicability of MICP for soil stabilization, crack repair in concrete, CO_2 sequestration, and heavy metal immobilization(Wuana and Okieimen, 2011).

Despite growing interest, limited studies have explored the potential of indigenous ureolytic metallotolerant bacteria for effective Pb^{2+} immobilization under environmentally relevant conditions. Furthermore, there remains a need to evaluate native isolates for their tolerance capacity, biomineralization efficiency, and practical remediation performance (Leeprasert, Chonudomkul and Boonmak, 2022).

Therefore, the present study aimed to isolate and characterize urease-producing metallotolerant bacteria from contaminated environments and to evaluate their potential for Pb^{2+} removal through MICP. The most promising isolate was identified at the molecular level and assessed for its calcium precipitation capacity and lead removal efficiency under optimized conditions(Han et al., 2023).

Although microbially induced calcium carbonate precipitation (MICP) has been widely studied for heavy metal remediation, most research has focused on model organisms such as Sporosarcina pasteurii under controlled laboratory conditions. Limited investigations have explored native ureolytic and metallotolerant bacteria from calcareous and industrial environments. Moreover, systematic evaluation combining urease activity, lead tolerance limits, multi-concentration Pb^{2+} removal efficiency, and mineralogical confirmation of biomineral formation remains insufficient. The potential of *Lysinibacillus fusiformis* as an effective ureolytic strain for Pb immobilization through MICP is still underreported, indicating the need for comprehensive investigation.

Material and Methodology:

Sample collection:

In July 2022, a total of six soil samples were collected from different locations in the Karad region (pH = 8.6, 30^{O}CTempreture = Latitude: 17.286501, Longitude: 74.181427), Satara district, Maharashtra, India, to isolate ureolytic metallotolerant microorganisms. Six samples were obtained from environmentally diverse sites within the Karad area, including agricultural fields (n = 2), riverbank soils along the Krishna River (n = 2), and urban/industrially contaminated soils (n = 2). Additionally, one soil sample was collected from an industrial area contaminated with heavy metals in Karad to assess the presence of metal-tolerant ureolytic bacteria. From each sampling site, approximately 500 g of soil was collected aseptically from a depth of 1–5 cm after removing surface debris. The samples were placed in sterile containers, transported to the laboratory in an ice box, and stored at 4 °C until further processing for bacterial isolation.. The selection of samples was done based on the presence of metal contamination and the presence of nitrogenous compounds, so that metal-tolerating ammonia-producing and calcium precipitating microbes can be obtained (Jalilvand et al., 2020).

Isolation of urease-producing bacteria from calcareous areas:

To isolate urease-producing bacteria from soil samples, 1 g of each sample was inoculated into 100 mL of urea broth medium (Sigma-Aldrich), which contained 1.00 mg/L peptone, 1.000 mg/L dextrose, 5.00 mg/L sodium chloride, 1.2 mg/L disodium phosphate, 0.8 mg/L monopotassium phosphate, 0.012 mg/L phenol red, and 6% (w/w) urea (sterile filtered 0.45 µm, added post-autoclaving) in 250 mL shake flasks and incubated at 30 °C for 120 h with shaking at 130 rpm. For further enrichment, 20% (v/v) of the culture was periodically transferred (up to three times) into fresh medium (Bibi et al., 2018).

The initial pH of the medium was adjusted to 7.0 ± 0.1 using sterile 1N NaOH or 1N HCl before inoculation. No external buffer system was used, as pH changes were intended to reflect urease activity during the experiment. The pH was monitored at regular intervals using a calibrated digital pH meter (Model Lab Hosp). After 72 h of incubation, the final pH increased to 8 ± 0.2. For isolation of bacteria, a 1 mL aliquot was serially diluted, and from the final enrichment, 0.1 mL of the enriched sample was spread onto urea agar plates using a sterilized L-shaped spreader until the liquid was evenly distributed. The plates were then incubated aerobically at 30 °C for 24 h. After incubation, colonies that showed a pink color were selected and purified by using nutrient agar. The spot was

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inoculated in triplicate on Christensen's agar. Urease production was assessed by observing color changes, with isolates showing ureolytic activity turning the urea agar from pale yellow to crimson red. The urease production test was studied through visual observation for color changes from yellow to crimson red. (Christensen, 1946). The diameter of the zone of crimson color around the colony (DZ) in mm, and the diameter of growth (DG), and then based on DZ and DG, the selection ratio was calculated. As DZ/ DG . The isolates showing maximum selection ratios were taken as the maximum urease producers. (Omoregie, 2016a). Potential isolates were further tested for lead tolerance.(Leeprasert, Chonudomkul and Boonmak, 2022)

Screening for the extent of metal tolerance of isolates:

The maximum tolerance concentration (MTC) of lead (Pb^{2+}) was determined using the agar plate dilution method. Nutrient agar plates were supplemented with Pb^{2+} (added as $PbCl_2$) at concentrations of 1, 2, 4, 6, 8, and 10 mM. Metal stock solutions were sterilized by membrane filtration (0.22 μm) and added to autoclaved medium after cooling to 45–50 °C.

Each isolate was spot-inoculated onto metal-amended plates in triplicate and incubated at 30 ± 2 °C for 7–10 days. Growth was monitored daily. The highest Pb^{2+} concentration permitting visible growth was recorded as the tolerance limit, whereas the lowest concentration completely inhibiting growth was considered the MTC.

Control plates without Pb^{2+} were maintained to confirm culture viability.(Rappazzo et al., 2025)

Screening of Calcium precipitation on solid medium:

The microbial isolate was screened for its calcium precipitation activity (CPA) by CPA assay techniques using the agar method. A modified method of Hammes et al.(Hammes et al., 2003) was adopted in this study and used to test the ability of the isolate to precipitate calcite. The Calcite precipitating medium (CPM) used in this study contained the following components: nutrient broth (3.0 g. L^{-1}); urea (20.0 g. L^{-1}); $NaHCO_3$ (2.12 g. L^{-1}); NH_4Cl (10.0 g. L^{-1}); $CaCl_2 \cdot 2H_2O$ (28.50 g. L^{-1}), and agar (20.0 g. L^{-1}). For Calcite precipitation screening, an overnight-grown isolate culture (OD600 ≈ 0.8) was serially diluted under sterile conditions and spread onto the CPM agar in triplicate. The Petri dish was then incubated at 30 °C for 6 days with the epidermal side facing upward. The precipitation zone around the growth was considered, as the organism was capable of calcium precipitation (Leeprasert, Chonudomkul and Boonmak, 2022).

Calcite precipitation was confirmed using a modified method from (Wei et al., 2015) . A nutrient broth, supplemented with 2% (w/v) urea and 2% (w/v) $CaCl_2$, was inoculated with culture grown overnight and then incubated at 30 °C with shaking at 150 rpm for 7 days. After incubation, the culture was centrifuged at 10,000 g for 60 seconds, and the resulting pellet was resuspended in 50 mL of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.5). Lysozyme at a concentration of 1 mg/mL was added, and the sample was incubated at 37 °C for 1 hour to facilitate cell lysis. The suspension was centrifuged again to remove cell debris, and the pellet was washed three times with distilled water (pH 8.5) at 4000 rpm. The purified $CaCO_3$ pellet was then air-dried at 37 °C for 24 hours, weighed to determine the calcite yield, and the dried powder was subsequently used for XRD analysis.

To qualitatively evaluate the calcium precipitation ability of bacterial isolates, calcium precipitation on a solid medium was examined. (Castro-Alonso et al., 2019). The appearance of visible calcium carbonate deposits around the colonies served as a sign of the potential for microbially induced calcium carbonate precipitation (MICP), allowing for the selection of effective strains for further studies focused on applications such as Bioprecipitation, Biomineralization etc (Albenayyan et al., 2023).

Media optimization for the removal of lead by UA6:

The optimal growth conditions for the promising ureolytic isolate UA6 were determined using a one-variable-at-a-time method. A culture of (UA6) that was 24 hours old was inoculated in triplicate into a minimal broth containing 1% (w/v) urea, and growth was observed by turbidity. The effects of various factors, including pH levels (5–10), temperature (30–50 °C), carbon sources (1% glucose, lactose, mannitol, and sucrose), nitrogen sources (1% peptone, tryptone, urea, ammonium chloride, and ammonium nitrate), incubation duration (24–96 h), and inoculum size (10^5–10^9 CFU mL^{-1}), were

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assessed separately. For each factor, the condition that resulted in the highest turbidity was considered as optimum conditions for the growth of UA6 (Williams, Kirisits and Ferron, 2016).

Removal of Lead (Pb) studies using a promising isolate UA6:

Five mL of a 48-hour bacterial culture (approximately 10^8 CFU/mL) were inoculated in triplicate into 45 mL of optimized MICP medium (Nutrient broth containing 2% urea + 2% Calcium chloride, final volume 50 mL) in 100-mL conical flasks. Flask was amended with 8 mM of heavy metal $Pb^{2+}(PbCl_2)$. Controls flask contains (medium + metal, no bacteria), Flasks were incubated at 28 °C with shaking at 200 rpm for 72 h. Samples (5 mL) were taken at 0, 24, 48, and 72 hours (if a time course was desired) and centrifuged at 8,000 × g for 15 minutes. Supernatants were filtered through 0.45-µm (or 0.22-µm) syringe filters, acidified to pH less than 2 with ultrapure HNO_3 for preservation, and stored at 4 °C until analysis(Omoregie, 2016b). Residual dissolved metal concentrations were measured using atomic absorption spectroscopy (AAS). Experiments were performed in biological triplicate; results are presented as mean ± standard deviation. Removal efficiency (%) was calculated as

$$\text{Removal Efficiency (\%)} = \frac{C0 - Ct}{C0} \times 100$$

Where:

C0 = Initial concentration of heavy metal in soil (mg/kg)

Ct = Final concentration of heavy metal after treatment (mg/kg)

Identification of isolate UA6:

(i) Based on morphological, cultural, and biochemical characteristics, the isolated organism was identified up to the generic level and some to the species level.

ii) By the 16S rRNA gene sequencing technique:

In order to identify the selected potential organism at the molecular level, the isolated pure organism was subjected to the 16S rRNA technique. A genome sequence was obtained, which was then used to generate a phylogenetic tree, and resemblance was used to identify the organism to the species level. This facility was hired from Sai Biosystems Private Limited, Nagpur.

A sequence similarity search was conducted using the BLAST tool to compare the obtained 16S rRNA sequence with existing sequences in the database. The highest aligned sequence from the BLAST search was used to identify the bacterial isolate. The BLAST tool.

Results and Discussion:

Sample collection:

Microbial isolates were obtained from a variety of environmental samples collected in the Karad region of Maharashtra, India (17.286501° N, 74.181427° E). These samples included calcareous soil, spent wash, tannery effluent, electroplating effluent, industrial wastewater, and cave samples. Each sampling location showed viable microbial growth, indicating the presence of metabolically active microorganisms adapted to their environments. In particular, the calcareous soil and cave samples showed dense microbial colonization, whereas industrial effluents supported the growth of stress-resistant isolates. The successful recovery of microorganisms from both natural and industrial sources underscores the ecological diversity of the area and gives a broad microbial pool for future screening of functional traits relevant to biomineralization and bioprecipitation applications.

Isolation of urease-producing bacteria from calcareous areas:

A total of sixteen ureolytic bacteria were isolated from environmental samples using media enriched with urea and then screened through spot inoculation on Christensen's urea agar. A variety of morphologically distinct isolates were obtained, among which nine were positive for urease production, which showed a color shift from pale yellow to crimson's red after incubation, indicating active urea breakdown. The level of urease activity differed among the isolates, as indicated by variations in the diameter of the crimson zone (DZ) and bacterial growth (DG). The calculated selection ratio (DZ/DG) was used to distinguish between strong and weak urease producers, with isolates showing higher ratios indicating greater enzymatic diffusion and activity. The alkaline

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condition observed is due to ammonia release during urea hydrolysis, a crucial process in carbonate formation. Isolates with significant urease activity are thus seen as promising candidates for microbially induced calcium carbonate precipitation (MICP) and related uses such as heavy metal immobilization and bioremediation. Selection ratios were calculated as

S/R =Diameter of zone in mm/ Diameter of colony in mm.

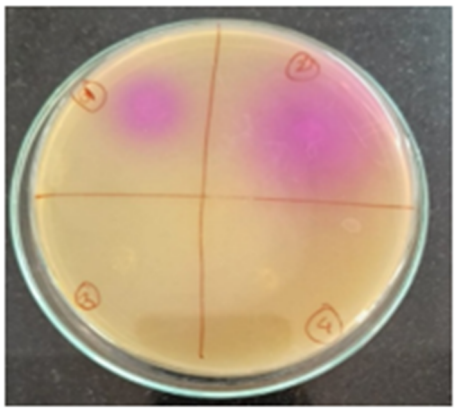

Figure 1. Urea hydrolysis zone by spot inoculation on urea agar, incubated at 30 °C/48 hr.

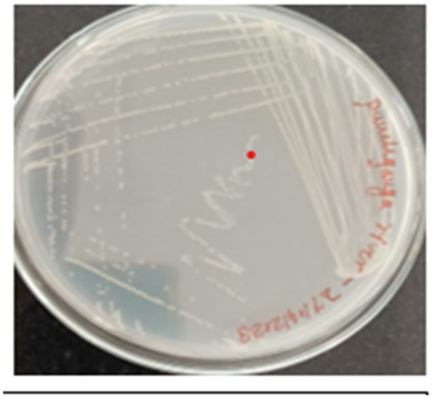

Figure 2. Growth of ureolytic, metal-tolerant UA6 isolate on nutrient agar incubated at 37 °C /48hr.

Table 1. Screening of urease producers.

Sr. no.	Isolate no.	Diameter of zone (mm)	Diameter of Colony (mm)	Selection ratio
1	UA1	1	1	1
2	UA2	2.5	1	2.5
3	UA3	–	–	–
4	UA4	–	–	–
5	UA5	3	1	3
6	UA6	4	1	4
7	UA7	–	–	–
8	UA8	2.5	1	2.5
9	UA9	–	–	–
10	UA10	3.5	1	3.5

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The selection ratio gave a comparative measure of extracellular urease production efficiency relative to the bacterial growth diameter, thereby serving as a reliable preliminary indicator for selecting potent ureolytic strains (Hammad et al., 2013; Wei et al., 2015).

It was cleared from the table and the fig among the tested isolates, UA6 showed the highest selection ratio (4.0), followed by UA10 (3.5) and UA5 (3.0), indicating strong urease producers. Moderate activity was observed in UA2 and UA8 (selection ratio 2.5), while UA1 gave a minimal S/R ratio (1.0). No detectable zone formation was recorded for UA3, UA4, UA7, and UA9.

Jalilvand et al., screened four isolates with the highest levels of urease production, calcite precipitation, and heavy metal, which were taken from contaminated sites. Zinc (Zn), lead (Pb), and cadmium (Cd) were bio-precipitated from solutions containing 100, 300, and 500 mM of these metals' ions using these isolates and *Sporosarcina pasteurii.* These bacteria's production of heavy metal precipitates was compared. The two isolates that formed the most carbonate minerals of heavy metals were *Variovorax boronicumulans* (C113) and *Stenotrophomonas rhizophila*(A323).

Screening for the extent of metal tolerance of isolates:

A total of nine isolates were screened for their metal tolerance using the MTC method. out of ten, only one showed metal tolerance to Lead up to 2mM to 8mM.

Nine ureolytic isolates (UA1–UA10) were tested for their capacity to increase levels of Pb^{2+}, ranging from 2 to 10 mM (Table 2). All isolates showed growth at 2 mM and 4 mM Pb^{2+}, indicating a basic level of tolerance that is often observed in environmental ureolytic bacteria exposed to moderately polluted areas. However, as the concentration of the metal Pb increased gradually, a growth of bacterial isolates was not observed. Then at 6 mM Pb^{2+}, only six isolates (UA2, UA4, UA5, UA6, UA7, UA8) were able to grow. Growth at 8 mM Pb^{2+} was given by one isolate, UA6, indicating that this strain has a higher tolerance to Pb^{2+} than the other isolates tested. UA6 was able to grow at 8 mM, which indicates the Maximum Tolerance Concentration. In contrast, isolates UA5, UA9, and UA10 showed the least tolerance to 4 mM Pb^{2+}. The differences in tolerance among the isolates may be due to variations in urease expression, metal efflux transporters, or biomineralization efficiency.

Table 2. Maximum Tolerance concentration of heavy metal (Pb) for isolate on nutrient agar supplemented with different concentrations of heavy metal (Pb).

	Different concentrations of Pb				
Isolate No.	**Pb (mM)**				
	2	4	6	8	10
UA1	+	+	–	–	–
UA3	+	+	+	–	–
UA4	+	+	+	–	–
UA5	+	+	+	-	–
UA6	+	+	+	+	–
UA7	+	+	+	–	–
UA8	+	+	+	–	–
UA9	+	+	–	–	–
UA10	+	–	–	–	–

Li et al., reported that the variation in Pb^{2+} removal ratios with increasing Ca^{2+} concentrations at different urea levels. When the Ca^{2+} concentration was 0.5 mol/L, the Pb^{2+} removal efficiency increased significantly compared to the tests without Ca^{2+}. Under these conditions, almost all Pb^{2+} ions were removed from the aqueous solution. When both urea and Ca^{2+} concentrations were 0.5 mol/L, the Pb^{2+} removal ratio reached 98–100%. Further increases in Ca^{2+} concentration resulted in a corresponding improvement in Pb^{2+} removal performance.

Calcium precipitation by UA6 isolate:

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UA6 showed both urease activity with metal tolerance up to 8Mm, then the UA6 potential isolate was subjected to calcium precipitation. The calcium carbonate precipitation ability of heavy metal-resistant and urease-producing bacteria was studied by using (calcium precipitation agar) on CPA agar, and a precipitation zone was observed surrounding the growth. Milky-white crystal was observed covering the colonies grown on the CPM and appeared at the 4th day of incubation. All the precipitates grown on the CPM appeared as a distinct circular zone around the growth area of the bacterial colony. This precipitation was primarily tested using 1 N HCl, poured on the growth, then effervescence was observed after adding the acid. It was observed that, after the isolate UA6 was treated with HCl, a precipitation zone with effervescence was formed. So, it was primarily concluded that UA6 has the capability for calcium precipitation. Then it was confirmed by XRD.

Sixteen bacterial isolates were obtained from the six soil samples. Among these, isolate UA6 showed the greatest increase compared with the other isolates and exhibited the largest precipitation zone. In contrast, isolate UA1 showed the smallest precipitation zone, with minimal bacterial growth.

Ekprasert et al., observed that the $CaCO_3$ precipitated in all bacterial culture flasks exceeded 0.1 g/L, whereas the non-inoculated controls exhibited precipitation of less than 0.02 g/L. This confirms that $CaCO_3$ formation resulted from microbial activity rather than purely chemical reactions.

The nature of crystals, crystallographic identity, and phase purity of inorganic compounds were determined using XRD. The characteristic signature peaks of calcite at 2h values of 24.13, 29.50, 34.04, 39.49, 44.31, 48.51, 49.65, 57.71, 58.50, 61.81, 62.22, 64.42, correlated with lattice (hkl) indices of (012), (104), (110), (113), (202), (024). The diffraction peaks of calcite matched with those of the standard spectrum JCPDS, No. 02-0629. JCPDS, No. 72-0506, respectively.

As per Eltarahony M, The characteristic signature peaks of calcite at 2h values of 23.13, 29.50, 36.04, 39.51, 43.31, 47.51, 48.65, 56.71, 57.50, 60.81, 63.22, 64.42, and 65.57, correlated with lattice (hkl) indices of (012), (104), (110), (113), (202), (024).(116), (211), (122), (214), (125), (300), and (0012), were identified in aerobic and anaerobic deposits of *L. sphaericus*and *R. planticola* (aerobically).

On the other hand, the reflection peaks of 20.93, 24.81, 27.13, 32.75, 39.82, 42.66, 43.13, 49.85, 51.13, 55.75, 60.36, 62.54 and 65.38 which corresponds to crystallographic planes of (002), (100), (101), (102), (103), (004), (110), (104), (200), (202), (105), (114) and (006), respectively, confirmed the presence of vaterite in anaerobic precipitates of *Raoultella sp.*and aerobic deposits of *Streptomyces sp.*. The diffraction peaks of calcite and vaterite matched with those of the standard spectrum JCPDS, No. 02-0629. JCPDS, No. 72-0506, respectively.

The XRD diffractogram revealed a significant peak around 29° (2θ), indicating that calcite is the primary $CaCO_3$ mineral present. This finding confirms the MICP activity of *Lysinibacillus*, showing its capability to produce highly crystalline $CaCO_3$ under the experimental conditions.

Removal of Lead by the best isolate UA6:

The Pb^{2+} removal efficiency of the MICP-positive isolate UA6 is presented in Table 3. A progressive decrease in residual Pb concentration (C_t) was observed over the 72-h incubation period. At 0 h, no removal was detected, as expected. After 24 h, the isolate achieved 50.77 ± 1.45% removal of the initial Pb^{2+} concentration, suggesting active ureolysis and initiation of carbonate precipitation during the early exponential growth phase.

Table 3. Metal removal capacity by UA6.

	R1	*R2*	*R3*	*Removal Efficiency (%) R1*	*Removal Efficiency (%) R2*	*Removal Efficiency (%) R3*	*Mean Removal (%)*	*SD*
0	415	415	415	0	0	0	**0**	**0**
24	210	198	205	49.4	52.3	50.6	**50.77**	**1.45**
48	125	118	122	69.9	71.6	70.6	**70.7**	**0.86**
72	65	58	62	84.3	86	85.1	**85.13**	**0.85**

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C_0 = initial concentration (t = 0), **C_t** = concentration after time **R1 → replicate, R2 → replicate 2**, R3 → **replicate 3.**

Table 4. Effect of Incubation Time on Pb^{2+} Removal Efficiency.

Incubation Time (h)	Removal Efficiency (%) (Mean ± SD)
0	0.00 ± 0.00
24	50.77 ± 1.45
48	70.70 ± 0.86
72	85.13 ± 0.85

Source of Variation	SS	df	MS	F-value	p-value
Between Groups	10963.27	3	3654.42	4425.6	< 0.0001
Within Groups	6.61	8	0.83		
Total	10969.88	11			

Pb^{2+} removal efficiency increased to 70.70 ± 0.86% at 48 h and further reached 85.13 ± 0.85% at 72 h. The time-dependent increase in removal efficiency indicates that sustained bacterial metabolic activity promotes $CaCO_3$ formation, facilitating Pb^{2+} immobilization through co-precipitation and/or surface adsorption mechanisms.

It was concluded from Table 3 and Figure 3 that the isolate UA6 is highly effective in removing Pb^{2+} within 72 hours. This level of performance highlights its potential for use in bioremediating lead-contaminated wastewater and supports its further application in MICP-based environmental remediation technologies.

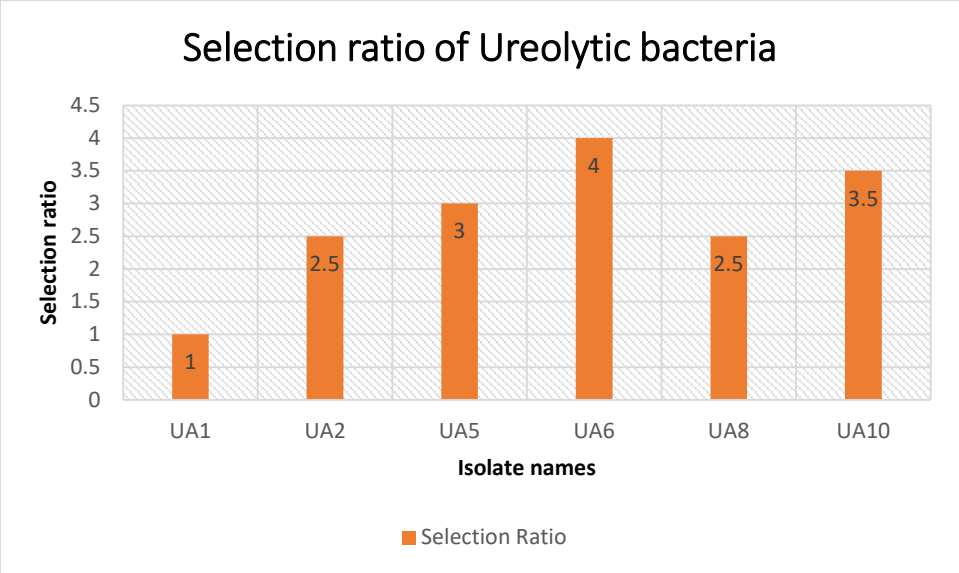

Figure 3. Selection ratio of ureolytic Isolates.

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A significant increase in Pb^{2+} removal efficiency was observed with increasing incubation time. Removal efficiency increased from 50.77 ± 1.45% at 24 h to 70.70 ± 0.86% at 48 h and reached a maximum of 85.13 ± 0.85% at 72 h. One-way ANOVA revealed a highly significant difference among incubation periods ($F = 4425.6$, $p < 0.0001$), indicating strong time-dependent biomineralization activity.

The low standard deviation values suggest high reproducibility and experimental consistency. The progressive increase in removal efficiency corresponds with enhanced urease activity and alkalization of the medium, facilitating calcium carbonate precipitation and subsequent Pb immobilization. This time-dependent removal trend is consistent with previously reported biomineralization behavior of the model MICP bacterium Sporosarcina pasteurii, where active metabolism significantly enhances heavy metal sequestration.

The observed removal efficiency of UA6 (85.13% at 72 h) is comparable to values reported for well-established ureolytic strains such as *Sporosarcina pasteurii*, which has been widely used as a model organism for MICP-based heavy metal immobilization. Previous studies have reported Pb^{2+} removal efficiencies ranging from 75–90% under similar laboratory conditions using *S. pasteurii*.

Similarly, other ureolytic members of the genus *Lysinibacillus sphaericus* have demonstrated Pb removal efficiencies between 60–85% depending on metal concentration and incubation period.

Although direct experimental comparison was not performed in the present study, the removal efficiency exhibited by UA6 falls within the range reported for these established MICP strains under controlled conditions. This suggests that UA6 possesses comparable Pb^{2+} immobilization capability at the tested concentration.

It is important to note that the present investigation was conducted using a single Pb^{2+} concentration (8 mM) under laboratory conditions. Therefore, while UA6 demonstrates promising Pb^{2+} removal performance comparable to standard ureolytic strains, further studies involving multiple metal concentrations, kinetic modeling, and side-by-side comparison with reference strains are necessary to comprehensively evaluate its relative efficiency.

Overall, the data support the potential of isolating UA6 as a candidate for MICP-mediated Pb^{2+} immobilization at the laboratory scale.

Mwandira *et al*., revealed the Microbially Induced Calcium Carbonate Precipitation (MICP) method by using the bacterium *Pararhodobacter sp*. for the bioremediation of lead. Laboratory-scale experiments were conducted, resulting in the complete removal of 1,036 mg/L of Pb2+.

According to Han *et al*.,(Han et al., 2023) The BS-based MIPP method, which is easily processed, has widespread applications in the remediation of heavy metal pollution. While it has been effective in removing Pb (II), there is a need for further exploration into its ability to eliminate other heavy metals. The method achieves Pb (II) immobilization by forming $Pb_5(PO_4)_3Cl$ precipitates within, on, and outside the cells. This precipitation process is driven by SGP chelation and the bacterial adsorption and accumulation of Pb (II).

16S rRNA gene sequencing identification:

At a molecular level, one potential bacterial isolate, viz., isolate UA6, was identified by analyzing the 16S rRNA gene sequence. BLAST was used to analyze the obtained sequences (Figure 4) of 16S rRNA genes of the potential isolate. The phylogenetic tree of isolates, indicating their relationship with other bacteria, was constructed by using the neighbor-joining approach. The percentage of bootstrap support is indicated by the numbers at the nodes. A phylogenetic tree was constructed using a bootstrap support value of 1000, which means the results are based on 1000 resampled datasets.

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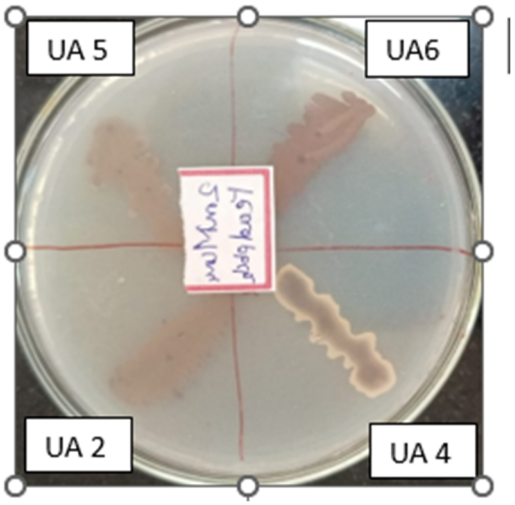

Figure 4. Isolate UA2, UA4, UA5, UA6 on nutrient agar supplemented with heavy metal (4mM lead chloride), incubated at ambient temperature for 7 days, with lead accumulated by isolates.

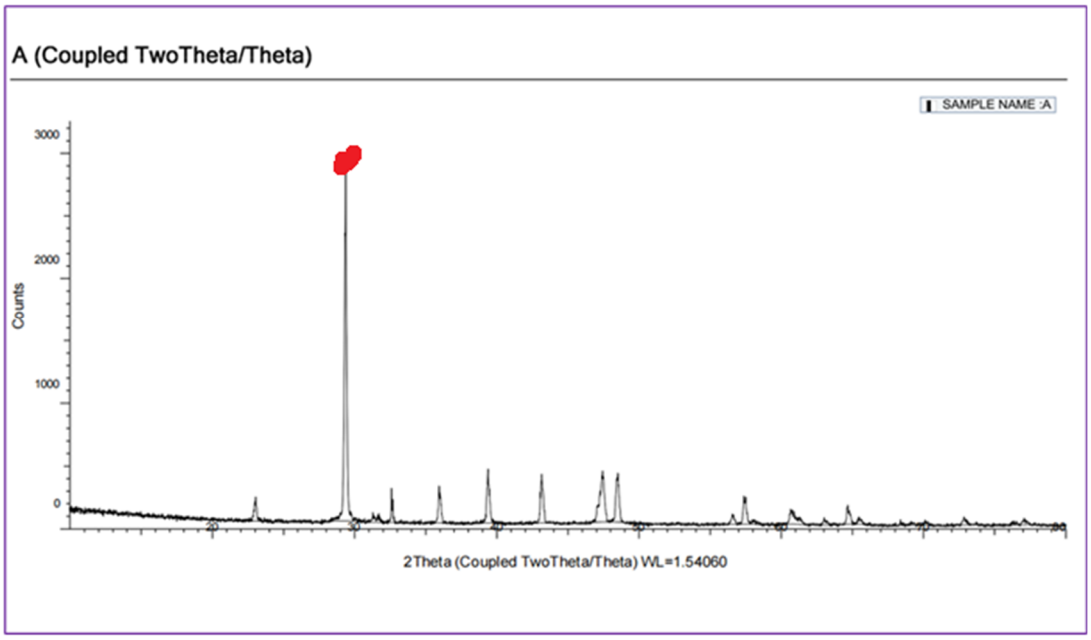

Figure 5. XRD profile of $CaCO_3$ crystals precipitated by promising isolate (UA 6).

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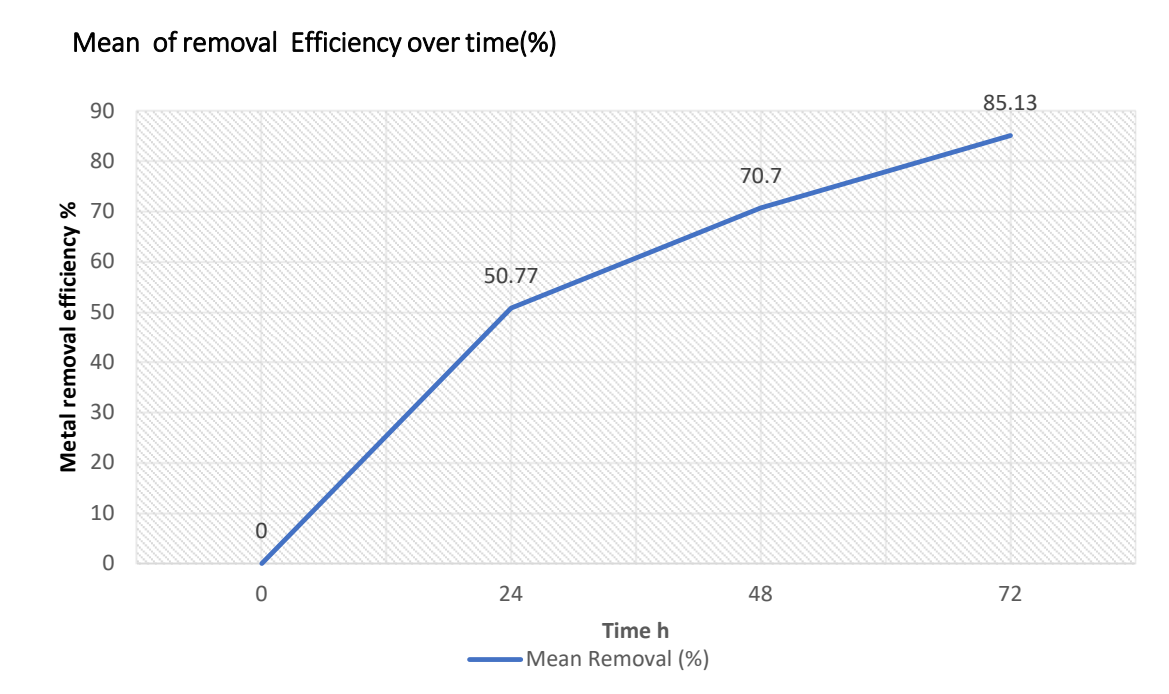

Figure 6. Metal removal efficiency by UA6.

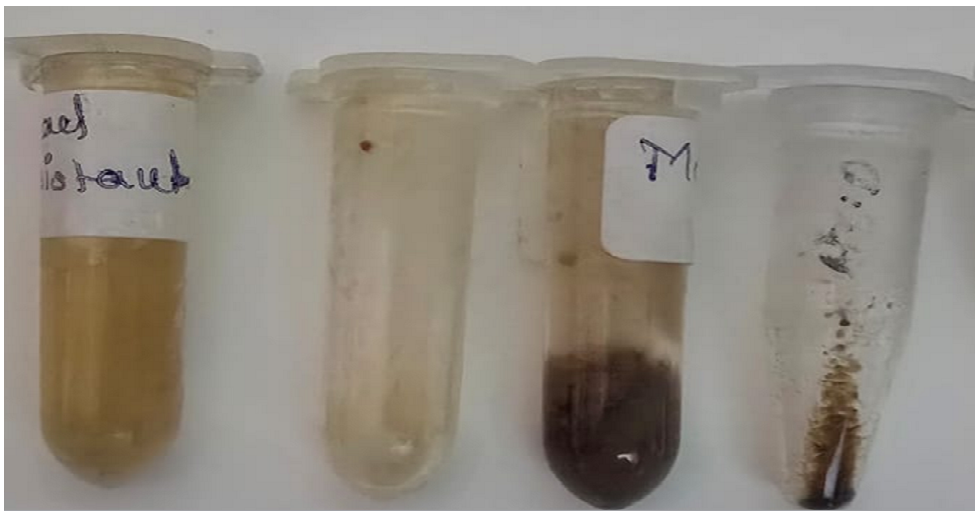

Figure 7. Visual appearance of samples prepared for AAS analysis for Pb removal experiments.

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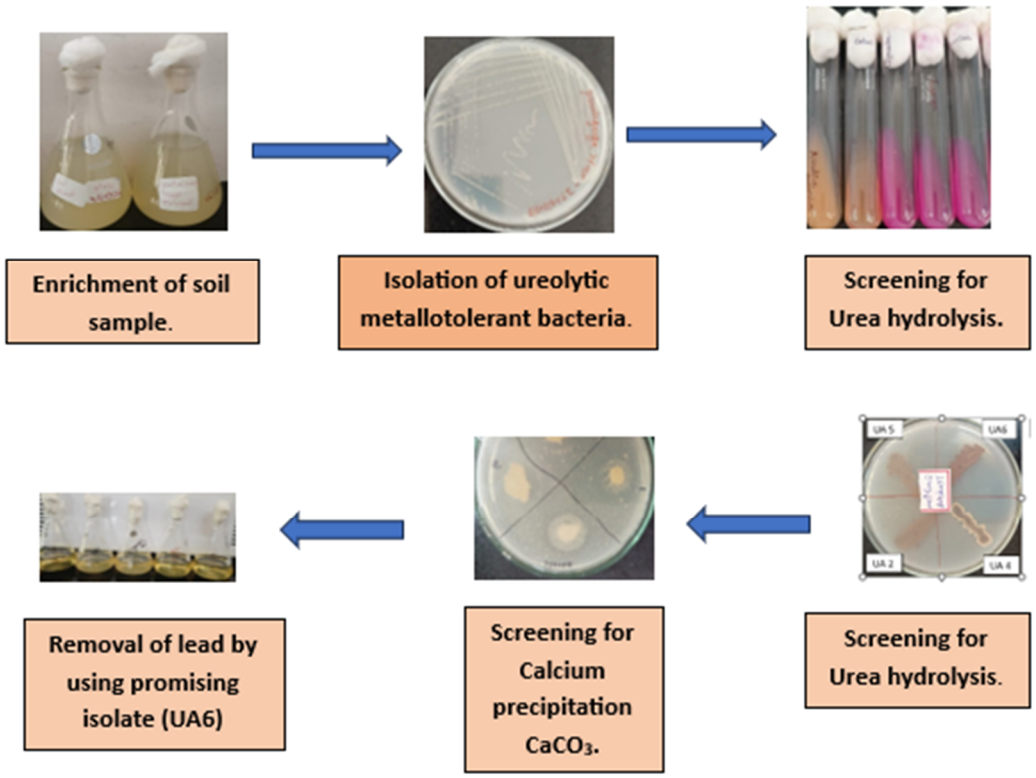

Figure 8. Graphical representation of experimental design.

The results revealed that isolate UA6 shared 99.88% sequence similarity with *Lysinibacillus fusiformis*. The obtained 16S rRNA gene sequence of isolate UA6 was submitted to the NCBI (National Center for Biotechnology Information) and identified as *Lysinibacillus fusiformis with* accession number PV203624.

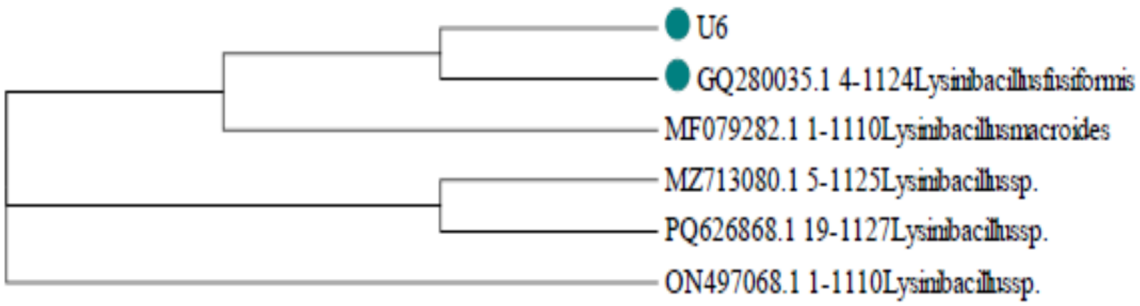

Figure 9. The phylogenetic tree for UA6.

Conclusion:

In A1. UA2, UA4, UA5, UA6, UA7, UA8, UA9, UA10) had potential for urea hydrolysis, and isolate UA6 was found to be the best Ureolytic metallotolerant calcium precipiting bacterium for lead removal. In future work, ureolytic, metallotolerant bacteria will be used for bio-cement production. Studies on the growth profiles of the isolate, CO_2 sequestration, optimization of growth factors, and biomineralization of heavy metals were recommended for future studies.

Future perspectives:

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The isolation of urease-producing bacteria from soil and their potential use in environmentally friendly bioremediation purposes. Future studies should focus on characterizing heavy metal carbonates by XRD and optimizing environmental conditions to enhance urease activity and microbially induced calcium carbonate precipitation in real-world settings. Transitioning from lab experiments to pilot and field-scale applications, along with assessing long-term stability and environmental safety, will be crucial. Developing microbial consortia and integrating MICP-based strategies with cost–benefit could further enhance their practical use for immobilizing heavy metals.

Ethical Approval: Not applicable.

Consent to Participate: Not applicable.

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