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Heavy metal immobilisation with microbial-induced carbonate precipitation: a review

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Microbial-induced carbonate precipitation (MICP) is a promising bioremediation technology for heavy metal immobilisation. This review explores the applications and efficacy of MICP in environmental challenges. It provides a comprehensive overview of the mechanism, primarily through ureolysis, detailing the process from urea hydrolysis to heavy metal precipitation as carbonate minerals. Alternative pathways such as photosynthesis and nitrate reduction are also discussed, highlighting the broad applicability of MICP. The review covers the historical evolution and advancements of MICP as a sustainable solution for heavy metal contamination. Recent studies demonstrate the efficiency of MICP in achieving high removal rates in diverse environments. The sustainable operation, precise targeting of heavy metal species, and versatility of MICP are examined. Challenges such as high copper concentrations, acidic conditions, and cost considerations are addressed. The article provides future directions and solutions to these challenges, including leveraging machine learning for optimal performance and enhancing cost considerations through detailed analyses. This review improves understanding of MICP's potential, provides a valuable resource for researchers in environmental engineering and the built environment, and encourages innovative approaches within these fields.

Keywords: biomaterials/biomineralisation/clean water and sanitation/cost-effective remediation/environmental impact/geochemical interaction/heavy metal remediation/microbial-induced carbonate precipitation/sustainability/UN SDG 6: clean water and sanitation

Introduction

The world faces an escalating challenge from environmental pollutants, including heavy metals contaminating soil, water, and ecosystems. Rapid industrialisation, human activities, and climate change have exacerbated this issue, resulting in significant health effects (Wang *et al.*, 2021). The consequences of environmental pollution are profound and extensive (Zhou *et al.*, 2022). Heavy metal contamination is a significant and pervasive environmental issue, posing a severe threat to environmental safety. These pollutants accumulate in soil, water, and air, entering the food chain and becoming more concentrated through biomagnification (Cui *et al.*, 2023), leading to severe health risks, including cancer, neurological disorders, and reproductive problems (Goswami and Neog, 2023). In addition, heavy metal pollution degrades agricultural soils, affecting food safety and quality.

The proportion of heavy metal pollution varies by industry, with mining contributing approximately 20%–40%, manufacturing 30%–50%, and agriculture 10%–20% (Su *et al.*, 2023). These estimates depend on industry type, location, and regulatory frameworks. For example, mining in developing nations may have higher pollution levels due to less stringent regulations, while agricultural pollution can vary with pesticide and fertiliser use (Mostafa *et al.*, 2023; Vácha, 2021). Specific pollution sources include ore extraction and waste management in mining, smelting and electroplating in manufacturing, and pesticides, fertilisers, sewage sludge, and livestock manure in agriculture (Vácha, 2021; Wang *et al.*, 2021; Zhou *et al.*, 2022). While industrial and natural activities contribute significantly to heavy metal pollution, it is critical to evaluate the effectiveness of current regulatory

frameworks and identify areas needing stricter enforcement or updated policies.

Mercury (Hg), arsenic (As), lead (Pb), and chromium (Cr) pollution are primarily from industrial and natural sources, accounting for 71.99%, 51.57%, 67.39%, and 68.36%, respectively (Cui *et al*., 2023). Cadmium (Cd) pollution is predominantly linked to agriculture, representing 84.12%. Reducing heavy metal pollution is essential to protect the environment and human health. Besides commercial sources and urban runoff, natural occurrences such as volcanic eruptions, floods, and landslides can disperse heavy metals, contaminating the environment and posing risks to human health (Yaashikaa and Kumar, 2022; Zhang *et al*., 2021). In urban areas, sources include lead paint, lead pipes, industrial emissions, landfills, and sewage sludge. Improper management of wastewater effluent from industrial or municipal sources can exacerbate this issue (Figure 1).

The clean-up and remediation of heavy metal pollution are costly. For instance, the estimated cost of addressing Pb contamination in Flint, Michigan, was US$2.1 billion (Mohammed *et al*., 2011). Healthcare costs for individuals exposed to heavy metals can be substantial, with the expense of treating a child with lead poisoning estimated at US$17 000 (Herath *et al*., 2022). Moreover, heavy metal pollution can result in lost productivity due to illness or disability, with annual costs reaching US$1.2 trillion (Wang *et al*., 2022a). Environmental damage from heavy metal pollution, impacting crop cultivation, livestock rearing, and fishing, is projected to cost approximately US$3.4 trillion annually. These figures underscore the need for cost-effective and efficient remediation methods to mitigate heavy metal pollution and its associated economic and health burdens.

Several techniques have been developed to address heavy metal pollution, including physicochemical adsorption using biochar and natural zeolite ion exchangers (Bai *et al*., 2023; Widiastuti *et al*., 2011), and the decomposition of heavy metal complexes through advanced oxidation processes and biogranulation (Basri *et al*., 2023; Nidheesh *et al*., 2022). However, these methods have significant limitations. Physicochemical adsorption may be constrained by the low availability of functional groups in biochar, reducing ion exchange (Hama Aziz *et al*., 2023). Advanced oxidation processes are costly and generate hazardous by-products, requiring proper treatment and disposal (Panwar and Pawar, 2022). Biogranulation is limited by the extended set-up time and is susceptible to environmental variations such as temperature and pH (Omar *et al*., 2023). The limitations of these methods highlight the need for innovative approaches that enhance remediation efficiency while minimising secondary environmental impacts.

Microbial-induced carbonate precipitation (MICP) is a biomineralisation process where microorganisms facilitate the formation of calcium carbonate ($CaCO_3$) deposits. MICP holds significant promise in bioremediation due to its ability to immobilise heavy metals and contaminants. The $CaCO_3$ crystals generated through MICP can encase contaminants, reducing their bioavailability and leaching into the environment (Yang *et al*., 2023). MICP is effective for remediating soil and water contaminated by heavy metals (i.e., Pb, As, and Cd). Despite its potential, the scalability and consistency of MICP applications in diverse environmental conditions require further research. Researchers have explored the use of MICP for restoring concrete structures affected by corrosion and chemical degradation (Achal *et al*., 2011a; Joshi *et al*., 2019). The potential of creating sustainable construction materials, such as self-healing concrete and bio-bricks, is also under investigation (Farajnia *et al*., 2022; Liu *et al*., 2021). Applications in soil biocementation, slope stabilisation, soil liquefaction, erosion control, and dust mitigation offer eco-friendly alternatives to conventional methods, which often involve high-energy inputs and synthetic chemicals (Gowthaman *et al*., 2022; Mwandira *et al*., 2019; Omoregie *et al*., 2024; Sun *et al*., 2021; Wang *et al*., 2022b). Compared with conventional grouting methods, microbial grouting using MICP techniques offers advantages such as reduced calcium consumption while maintaining the same compressive strength (Naeimi and Haddad, 2020). These findings underscore the potential of eco-friendly alternatives in soil improvement.

MICP has wide-ranging applications in bioremediation and construction, making substantial contributions to environmental and construction sustainability. Its efficacy has garnered significant attention, leading to a surge in research and recognition as shown in Table 1. The number of publications on MICP increased from two in 1990 to 210 in 2023, with citations rising to 9513 (see Figure S1). This growth reflects MICP's expanding scope, potential to address environmental and construction challenges and significance in shaping innovative solutions. Researchers and professionals increasingly recognise the value of MICP for heavy metal removal, a trend expected to continue as MICP advances and proves its effectiveness in practical applications. MICP is particularly relevant for heavy metal contamination.

This review provides a comprehensive overview of the mechanisms of MICP and factors influencing heavy metal removal. It investigates the conceptual evolution of MICP, covering historical aspects, advancements, and research hotspots. In addition, the review addresses safety practices and sustainable approaches, such as environmental safety, optimising remediation performance, and sustainable soil improvement. Challenges in MICP efficiency, including high copper (Cu) concentration, acidic conditions, environmental impact assessment (EIA), and cost considerations, are discussed. Furthermore, the review offers potential future directions for MICP research, including reducing Cu toxicity, utilising acid-tolerant microbes, ensuring long-term stability, enhancing cost considerations, and leveraging machine learning.

Figure 1. Improper drainage blockage issue in a commercial area, highlighting the presence of potential unwanted pollutants if not properly treated

Table 1. List of various heavy metals immobilised using MICP techniques

Contaminated material	Ureolytic microorganism and source	Source of microbe	MICP treatment method	Crystal identification or precipitated	Removal performance of heavy metal	Reference
Pb-contaminated saline solution in reactor	*Exiguobacterium* sp. JBHLT-3	Mongolia, China	*Exiguobacterium* sp. was tested for $CaCO_3$ precipitation in saline conditions, including Pb-amended bioprecipitation experiments at diverse salinities (3%–12%). Soluble Pb removal was evaluated in specific reactor conditions during a 5-day incubation at 25°C.	Calcite and vaterite	96.7% removal of Pb^{2+}	(Bai *et al.*, 2021)
Cd-contaminated solution	*Serratia marcescens* NCIM 2919 and *Enterobacter cloacae* EMB19	Maharashtra and Delhi, India	Bacterial cultures were grown in 125 mL of nutrient medium with 2% urea. After 72 h, cell-free supernatants were collected for in vitro Cd(II) remediation. Each supernatant was mixed with 25 mM $CaCl_2$ and/or 50 mg/L Cd(II), incubated for 12 h, and then centrifuged. The residual Cd(II) and/or Ca(II) levels were measured, and the resulting Cd–Ca composites were dried and characterised.	Calcite	79%–98% removal of Cd(II)	(Bhattacharya *et al.*, 2018)
Sulfide-bearing tailings	*Sporosarcina luteola*	Guerrero, Mexico	For metal and metalloid resistance assessment, *Sporosarcina luteola* (1.08×10^8 CFU/mL) was cultured on pH 9.0 ATCC Medium 1376 plates with 0.05–50 mM of various compounds. The MIC for growth inhibition was determined.	Calcite, vaterite, rhodochrosite, cerussite, otavite, strontianite, hydrozincite, witherite, and hydromagnesite	Not declared	(Cuaxinque-Flores *et al.*, 2020)
Pb–zinc tailings samples	*Sporosarcina pasteurii*	Not declared	An acrylic pipe mould (50 mm inner diameter, 170 mm height) coated with petroleum jelly facilitated a layered curing process, gradually adding lead and zinc tailings (1–10 cm) daily until exceeding 11 cm. The injection of 150 mL of *Sporosarcina pasteurii* solution was followed by 150 mL of cementing solution after draining the bacterial solution. After a 2-day maintenance period, specimens were de-moulded and dried.	Aragonite, calcite, vaterite	Fe^{3+}, Zn^{2+}, Pb^{2+}, Cu^{2+} and Cd^{2+} in the leaching solution were completely removed (100%), while Mn^{2+} and Cr^{3+} were removed up to 98.24% and 95.56%, respectively.	(Dong *et al.*, 2023b)
Contaminated water	*Metschnikowia pulcherrima* and *Raoultella planticola*	Alexandria, Egypt	Various Pb^{2+} and Hg^{2+} concentrations (700, 350, 175, 80, 40, 20, and 10 ppm) were assessed. In 500 mL flasks, 150 mL of mineralisation media with 350 ppm (1/2 minimum inhibitory concentration) of Pb^{2+} and Hg^{2+} was inoculated with 108 CFU/mL of each microbial strain. Incubation took place in a rotary shaker (150 rpm) at 30°C for 7 days, with abiotic controls concurrently incubated without microbial inoculum.	Calcite, vaterite, calcium lead oxide, calcium mercury(II) oxide, and mercury(II) oxide	Not declared	(Eltarahony *et al.*, 2021b)

(continued on next page)

Table1. Continued

Contaminated material	Ureolytic microorganism and source	Source of microbe	MICP treatment method	Crystal identification or precipitated	Removal performance of heavy metal	Reference
Cu–Ni-contaminated tailing samples	*Lysinibacillus fusiformis* strain Lf	Zurich, Switzerland	Cu, Ni, and Cr exhibited elevated concentrations exceeding 600 mg/kg, with Pb^{2+} measured at 32 ± 9 mg/kg. Tailings solidification was monitored in 150 × 150 × 150 mm^3 moulds over 90 days. Each mould received 5 kg of tailings, 1.5 L of bacterial culture (1% v/v bacterial solution, 2% w/w urea), and 250 g of CaO in relevant groups. Control groups used deionised water instead of bacterial culture. CaO was added after 24 h of bacterial incubation, and the mixture solidified in a greenhouse (25°C, 50% humidity) for 90 days.	Calcite, albite, and cordierite	The long-term passivation rates were 78.8% for Cu, 78.1% for Ni, 89.2% for Pb, and 97.8% for Cr.	(He *et al.*, 2023)
As-contaminated soil	*Leptolyngbya* sp. XZMQ and *Bacillus* sp. XZM	Wuhan and Shanxi, China	Varied concentrations (0–1600 mg/L) of As(III) and As (V) were examined. Eighty grams of soil, collected near the Shimen Realgar tailings, was placed in a Petri dish (90 mm diameter, 15 mm depth). A suspension of bacteria or microalgae (15 × 10^5 cells) was evenly inoculated onto the soil. Plates were incubated for 45 days at 25°C (dark: light cycle 10:14 h), with water replenished every 3 days based on weight loss, and 1 mL (10% BG-11:10% LB, 1:1) medium added every 5 days.	Not declared	As(III) and As(V) in the soil were immobilised up to 26%.	(Mao *et al.*, 2023)
Cd-contaminated water and soil	*Enterobacter* sp.	Sichuan Province, China	Different concentrations (20, 40, 60, 80, 100 mg/L) of Cd were investigated. Cd removal ability was initially assessed in liquid medium using LB medium (100 mL) with varying Cd concentrations (20–100 mg/L) and pH (4–9). Flasks in the MICP group contained urea (2%), $CaCl_2$ (40 mM), and bacterial suspension (1%, OD600 = 0.5). Samples were collected on the 7th day to measure the Cd concentration. In soil incubation experiments, eight treatments were tested in 1000 mL beakers with 0.5 kg Cd-contaminated soil. Oyster shell wastes (2%), bacterial suspension (50 mL, ~10^9 CFU/mL), and urea (1%) were added to corresponding groups.	Vaterites and calcites	Cd-immobilising rate in water and soil reached 99.50%, and 56.10%, respectively.	(Peng *et al.*, 2020)
Fe-tailings slag backfill	*Sporosarcina pasteurii* BNCC 337394 strain	Not declared	Fe-tailings slag, sieved through a 0.75 mm sieve, was mixed with water and cementitious materials in a 0.4 water–solid ratio. The resulting mixture underwent grinding, was placed in moulds, injected with a bacterial solution, and	Silicon dioxide, iron(III) oxide, ettringite, and $CaCO_3$	Not declared	(Qiu *et al.*, 2022)

(continued on next page)

Table1. Continued

Contaminated material	Ureolytic microorganism and source	Source of microbe	MICP treatment method	Crystal identification or precipitated	Removal performance of heavy metal	Reference
			termed microbial-cemented iron tailings–based backfill. Curing times of 3 and 7 days were selected based on strength requirements.			
Heavy metals contaminated soil	*Sporosarcina pasteurii* ATCC 11859 strain	Purchased from the National Collection of Industrial Microorganisms	Pb, Zn, and Cr(VI) stock solutions at concentrations of 1000, 2000, 3000, 4000, and 5000 mg/L were used. The sand was contaminated with Pb, Zn, and Cr(VI). Sand was mixed with metal solutions, air-dried overnight, saturated with water, and incubated for uniformity. Cylindrical PVC moulds formed specimens with specified conditions. Bacterial suspension was added for 24 h, repeated on the 6th and 12th days in metal-treated sands. After draining, the cementation solution was added. Fresh cementation solution was replaced every 12 h for 16 days (except the 6th and 12th days).	Calcite	Pb and Cr(VI) immobilisation up to 92% and 94%, respectively, in contaminated sand. However, Zn was found highly toxic to *S. pasteurii*	(Zakrzewska *et al.*, 2023)
Cd-contaminated soil	*Ochrobactrum* sp. POC9	Warsaw, Poland	Various concentrations (50, 500, 5000, and 10000 mg/kg) of Cd were employed. Garden soil was commercially obtained and artificially contaminated with Cd to simulate natural agricultural conditions. Cadmium chloride solution was added to achieve a final Cd concentration of 2 mg/kg in dry-weight soil. The soil was thoroughly mixed and incubated at room temperature with periodic watering to maintain 50% humidity. Mixing every 3 days ensured homogeneity. Soil conditioning with Cd occurred over 30 days before being subjected to MICP treatment with different concentrations of *Ochrobactrum* sp.	Not declared	Reduction of the Cd bioavailability ranged from 28% to 65% in the soil.	(Zhang *et al.*, 2023)
Pb-contaminated nutrient solution	*Brevibacillus laterosporus* ZN5	Jilin Province, China	Various concentrations (50, 80, 100, 120, 150 mg/L) of Pb(II) were investigated. A 4 mL bacterial cell suspension (2.1×10^8 CFU/mL) was inoculated into 200 mL growth media. The cultures were then incubated at 30°C and 160 rpm for 120 h to create the bacterial fermentation suspension. Subsequently, the bacterial fermentation suspension was enriched with a 15 mM Pb(II) solution. Samples were collected at 20 min, 1 h, 3 h, 8 h, and 18 h, and the upper solutions were filtered through a 0.45 μm membrane to determine the Pb(II) concentration.	Hydrocerussite and lead carbonate	Ammonification and nitrate assimilation processes exhibited rapid removal of Pb(II), achieving maximum removal efficiencies of 94% and 70%, respectively	(Zhao *et al.*, 2020b)

MICP mechanism for heavy metal removal

Overview of MICP

MICP is a biogeochemical process where microorganisms produce minerals at low energy costs, offering innovative possibilities in engineering applications (Murugan *et al*., 2021). Among various types of biomineralisation, MICP is classified as biologically induced mineralisation, where microorganisms alter the pH of their surroundings, leading to carbonate precipitates (Bisht *et al*., 2020; Power *et al*., 2007; Zhi *et al*., 2014). While microorganisms do not directly control mineral deposit formation, they influence the process indirectly through environmental pH changes. Understanding the interplay of physicochemical and biological factors is crucial for optimising MICP efficiency, with bacterial cell concentration being a key factor (Murugan *et al*., 2021).

In the MICP endeavours for heavy metal removal, the typical procedure encompasses several key steps. It commences with the preparation of ureolytic bacterial cultures and the necessary sterilisation of reagents, chemicals, and media. While conventional factors such as biomass concentration/viability, pH, and urease activity are monitored, precipitation tests are routinely executed to gauge the formation of carbonate precipitates and appraise the resilience of bacterial cells against heavy metal ions present in the solution or medium (Xiao *et al*., 2021). To showcase the transformative potential of soil biocementation, the MICP process is applied, occasionally even in a liquid solution. Advanced analytical techniques are employed to scrutinise the MICP process and its impact on the treated materials. Furthermore, the structural integrity and durability of materials treated through MICP are evaluated using an unconfined compressive strength machine. Understanding MICP mechanisms is essential for leveraging its potential in heavy metal immobilisation (Figure 2). This includes exploring the preferred ureolysis pathway and alternative MICP pathways, highlighting their significant potential for heavy metal remediation.

Ureolysis pathway for MICP

Ureolysis, or urea hydrolysis, is the most common pathway for MICP, primarily facilitated by urease-producing bacteria such as

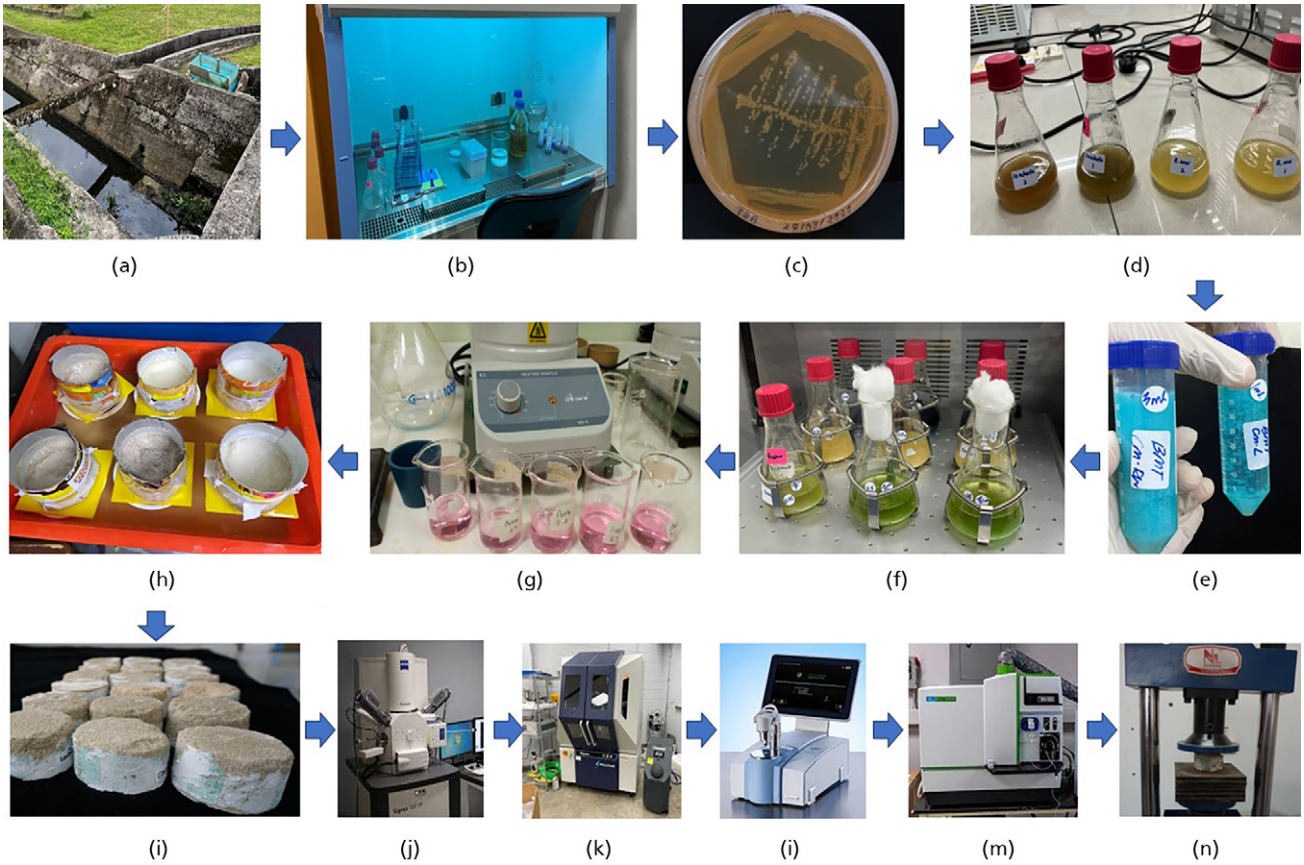

Figure 2. MICP treatment for heavy metal sequestration. (a–d) MICP set-up and preparation of ureolytic bacterial cultures; (e–g) precipitation tests, including the quantification of carbonate precipitates and bacterial cells undergoing tolerance tests in the presence of heavy metals; (h–i) process of soil biocementation by way of MICP, both before and after curing; (j–m) various analytical methods for material characterisation; and (n) the strength testing procedure using an unconfined compressive strength machine

Sporosarcina pasteurii and *Lysinibacillus sphaericus*. These bacteria hydrolyse urea using the extracellular urease enzyme (Meier *et al*., 2017). The hydrolysis process elevates the pH, promoting $CaCO_3$ precipitation from calcium and bicarbonate ions in solution. This pH elevation results from the consumption of organic compounds, producing carbonate ions (CO_3^{2-}) and hydroxide ions (OH^-) as by-products (Omoregie *et al*., 2022). The resulting alkaline environment is essential for the subsequent precipitation of $CaCO_3$.

Microorganisms play a dual role in biomineralisation. First, they create an environment conducive to carbonate mineral formation by elevating the local pH through their metabolic processes (Xiao *et al*., 2021). Second, they produce extracellular polymeric substances (EPS), complex molecules serving as a matrix for Ca^{2+} and CO_3^{2-} ions to aggregate, forming initial mineral nuclei (Li *et al*., 2017). EPS not only initiate mineral formation but also act as an effective immobilising agent for heavy metals. Heavy metal ions bind to the EPS and integrate into the $CaCO_3$ structure, significantly reducing their mobility and bioavailability (Huang *et al*., 2018).

The chemical reactions involved in heavy metal removal through MICP include actions by urease and carbonic anhydrase enzymes. The process starts with urea ($CO(NH_2)_2$) reacting with water (H_2O) in the presence of urease, producing ammonia (NH_3) and carbamic acid (NH_2COOH) (Jing *et al*., 2023; Qiao *et al*., 2021). Urease catalyses this hydrolysis, breaking down urea (Equation 1). The produced NH_2COOH further reacts with H_2O to form carbonic acid (H_2CO_3) and additional NH_3 (Equation 2). H_2CO_3 then dissociates into (H^+) and bicarbonate ion (HCO_3^-), facilitated by carbonic anhydrase, increasing bicarbonate ion concentration (Equation 3).

Next, NH_3 reacts with H_2O to generate OH^- and ammonium ions (NH_4^+) (Equation 4), contributing to the pH increase around the microbial cells. The HCO_3^-, generated in Equation 3, interacts with H^+ and additional OH^- to produce CO_3^{2-} (Equation 5). These carbonate ions precipitate from the solution when exposed to divalent cations. Calcium ions (Ca^{2+}) in the microbial environment can react with CO_3^{2-} to form solid $CaCO_3$ precipitates (Equation 6). Similarly, heavy metal ions (HM^{2+}) present in the solution can react with CO_3^{2-} to produce solid heavy metal carbonates ($HMCO_3$) (Equation 7). In certain scenarios, $HMCO_3$ co-precipitates with $CaCO_3$ to form mixed carbonate minerals, where 'x' represents the proportion of heavy metal ions in the precipitate (Equation 8). Figure 3 provides a schematic representation of MICP, leading to the removal of heavy metals and the simultaneous co-precipitation of $CaCO_3$. The corresponding reaction equations are presented below:

1. $$CO(NH_2)_2 + H_2O \rightarrow NH_3 + NH_2COOH$$

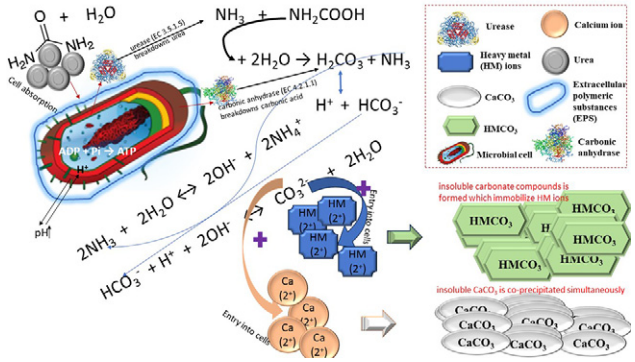

Figure 3. Schematic illustration showing the MICP mechanism for heavy metal immobilisation and co-precipitation of $CaCO_3$

2. $$NH_2COOH + H_2O \rightarrow H_2CO_3 + NH_3$$

3. $$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

4. $$2NH_3 + 2H_2O \leftrightarrow 2OH^- + 2NH_4^+$$

5. $$HCO_3^- + H^+ + 2OH^- \rightarrow CO_3^{2-} + 2H_2O$$

6. $$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_{3(g)}$$

7. $$HM^{2+} + CO_3^{2-} \rightarrow HMCO_{3(g)}$$

8. $$xHM^{2+} + (1-x)Ca^{2+} + CO_3^{2-} \rightarrow Ca_{(1-X)}M_x\ CO_{3(g)}$$

Urease, a large enzyme complex with a molecular mass exceeding 500 kDa, contains two nickel (Ni) ions vital for its catalytic function (Zhu *et al*., 2021). This enzyme is essential for breaking down urea into carbonate ions, which then react with calcium ions to form $CaCO_3$ precipitates, effectively trapping and immobilising heavy metals. The Ni ions within the enzyme's active site are indispensable for catalytic activity, a prerequisite for carbonate ion production. Carbonate ions subsequently react with calcium ions to generate $CaCO_3$ precipitates, effectively trapping and immobilising heavy metals as demonstrated in Figure 3. The intricate molecular mechanism governing urease catalysis is yet to be fully elucidated, but it has played a fundamental inspiration in heavy metal immobilisation.

Ureolysis is preferred in MICP due to its simplicity, the abundance of urease-producing bacteria, and the rapid pH elevation from liberated ammonia (Lauchnor *et al*., 2015). These bacteria, such as *Sporosarcina aquimarina*, *S. pasteurii*, and *Exiguobacterium undae*, adapt well to various environmental conditions, including alkaline pH and high calcium concentrations (Keykha *et al*., 2019; Kumari *et al*., 2014). This knowledge forms a solid foundation for enhanced control and predictability within MICP processes. The accessibility and affordability of urea also make MICP economically viable and scalable (Chen *et al*., 2019; Omoregie *et al*., 2019a). The extensive knowledge of urea hydrolysis and $CaCO_3$ precipitation by these bacteria provides valuable insights for optimising the MICP process

Other MICP pathways

MICP can occur through various metabolic pathways (apart from ureolysis) through microorganisms, each contributing to mineral precipitation. These distinct mechanisms include (1) photosynthesis, (2) ammonification, (3) nitrate reduction (denitrification), (4) sulfate reduction, and (5) iron reduction. Each of these pathways contributes to mineral precipitation through different mechanisms.

First, oxygenic photosynthesis unfolds as a captivating process. Specific microorganisms, exemplified by cyanobacteria (i.e., *Synechococcus elongatus*), and algae (i.e., dinoflagellates) harness the radiant energy of sunlight to orchestrate a transformation (Bundeleva *et al*., 2014; Frommlet *et al*., 2015). This transformation sees carbon dioxide (CO_2) and water metamorphose into organic matter while simultaneously liberating oxygen as a noteworthy by-product (Equation 9). The exchange of HCO_3^- or OH^- ions lead to an elevation in alkalinity across microbial cells (Lamérand *et al*., 2022). What makes this process especially intriguing is the ability of these microorganisms to influence their immediate surroundings. Through the consumption of CO_2, they manipulate pH levels, setting the stage for the precipitation of $CaCO_3$ (Kawaguchi and Decho, 2002). This biomineralisation process is set into motion as conditions shift to favour $CaCO_3$ formation. Increased alkalinity from the photosynthesis process facilitates the reaction between Ca^{2+} and HCO_3^-, leading to the formation of $CaCO_3$ minerals (Equation 10).

9. $$CO_2 + H_2O \rightarrow Organic\ matter + O_2$$

10. $$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + O_2$$

During ammonification, microorganisms convert organic nitrogen-containing compounds, such as proteins and amino acids, into NH_3 (Equation 11). The generated NH_3 elevates the pH levels in the vicinity. The generated NH_3 exhibits the remarkable capability to elevate the pH levels in the vicinity. Then, NH_3 react with HCO_3^- to form NH_4^+ and CO_2 (Equation 12). The subsequent reactions with calcium ions lead to the precipitation of $CaCO_3$ (Equation 13), a key development in the MICP journey. While some bacteria, such as *Virgibacillus marismortui*, acidify their environment (Zhao *et al*., 2020b), others, such as *Brevibacillus laterosporus*, raise the pH through ammonification (Gunes and Balci, 2021). This increase in pH, observed in various species (i.e., *Rheinheimera texasensis*, *Paeniglutamicibacter kerguelensis*, *Ensifer adhaerens*, *Microbacterium testaceum*, and *Pseudomonas protegens*) (Hatayama and Saito, 2019), promotes the precipitation of $CaCO_3$, a key step in MICP.

11. $$Protein\ or\ amino\ acid \rightarrow NH_3$$

12. $$NH_3 + HCO_3^- \rightarrow NH_4^+ + CO_2$$

13. $$NH_4^+ + Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + 2H_2O + NH_3$$

Denitrifying bacteria contribute to MICP through a distinctive pathway. These bacteria use nitrate (NO_3^-) as a terminal electron acceptor, and in the process, they generate nitric oxide (NO) (Equation 14). NO, in turn, can react with calcium ions, forming calcium nitrate ($Ca(NO_3)_2$) (Liu *et al*., 2022a). This metabolic feat leads to an intriguing pH elevation outcome. As the pH undergoes this shift, it lays the foundation for the precipitation of $CaCO_3$, an essential aspect of MICP. For instance, calcium nitrate is hydrolysed, leading to the precipitation of $CaCO_3$ and the release of nitric acid (Equations 15 and 16).

14. $$NO_3^- \rightarrow NO$$

15. $$Ca^{2+} + 2NO_3 \rightarrow Ca(NO_3)_2$$

16. $$Ca(NO_3)_2 + 2H_2O \rightarrow CaCO_3 + 2NO_3^- + H_2O_2$$

Halomonas sp. and *Thauera* sp. are reported microbial species known for their versatile metabolism and ability to form biofilms (Chetty *et al*., 2023). This process is not well understood, especially at alkaline pH levels. Researchers observed a decrease in nitrate reduction rates from 0.72 mM/h at pH 9.5 to 0.17 mM/h at pH exceeding 11, while the concentration of nitrite increased (Chetty *et al*., 2023). The pH shift sets the stage for the precipitation of $CaCO_3$, which plays a key role in MICP.

The denitrification-driven pathway demonstrates the adaptability of the process (Pham *et al*., 2018).

Sulfate reduction is a pivotal process contributing to sulfide generation in environments with high sulfate concentrations and low oxygen levels, both in natural and industrial contexts (Ren *et al*., 2022). Certain sulfate-reducing microorganisms (i.e., *Desulfovibrio* sp.) induce MICP by producing sulfide ions (S^{2-}). In this metabolic pathway, microorganisms pivot towards sulfate (SO_4^{2-}) as a terminal electron acceptor during anaerobic respiration (Karnachuk *et al*., 2021). Then, sulfate later converts into S^{2-} by way of a transformational phase (Equation 17). These sulfide ions then react with Ca^{2+} in the environment, resulting in the formation of calcium sulfide (CaS) (Chetty *et al*., 2023; Gao *et al*., 2023). Subsequently, this calcium sulfide can be oxidised, typically by atmospheric oxygen or other oxidants, leading to the conversion of CaS into $CaCO_3$ and elemental sulfur (S) (Equations 18 and 19).

17. $$SO_4^{2-} \rightarrow S^{2-}$$

18. $$Ca^{2+} + S^{2-} \rightarrow CaS$$

19. $$CaS + O_2 \rightarrow CaCO_3 + S$$

Iron reduction unfolds as a fascinating metabolic pathway where microorganisms channel the potential of ferric iron (Fe^{3+}) as an electron acceptor during anaerobic respiration. Iron-oxidising bacteria such as *Sideroxydans* sp., *Gallionella* sp., and *Azoarcus* sp. play a role in MICP by reducing (Fe^{3+}) to ferric iron (Fe^{2+}) (Equation 20) (Levett *et al*., 2020). The produced ferric iron interacts with HCO_3^- in the environment, forming ferric hydroxide ($Fe(OH)_3$) and CO_2 (Equation 21) (Yang *et al*., 2022). This ferric hydroxide subsequent transformation leads to the precipitation of $FeCO_3$ and H_2O (Equation 22). The iron-mediated pathway adds another layer of complexity to the MICP process (Ning *et al*., 2022). Meanwhile, the release of CO_2 occurs during the initial reaction. The precipitation of $CaCO_3$ is not explicitly shown in this pathway, as $FeCO_3$ is the primary product in this case. Microbial iron reduction influences pH by balancing proton (H^+) consumption during iron reduction and (H^+) generation from organic substrate oxidation (Li *et al*., 2022a). In Fe^{3+}-rich conditions, it increases pH, converting dissolved CO_2 into bicarbonate. Beyond urea hydrolysis, MICP pathways can immobilise heavy metals such as Pb, Cd, and Cu by forming less soluble compounds. This adds complexity to MICP, which influences the pH and contributes to $FeCO_3$ formation.

20. $$Fe^{3+} + e^- \rightarrow Fe^{2+}$$

21. $$Fe^{2+} + HCO_3^- \rightarrow Fe(OH)_3$$

22. $$Fe(OH)_3 \rightarrow FeCO_3 + H_2O$$

Factors influencing MICP effectiveness

The effectiveness of heavy metal immobilisation through MICP is governed by a multitude of factors. Understanding these factors is essential for optimising this bioremediation strategy. By critically analysing these elements, the application of MICP can be enhanced in diverse contaminated environments, ensuring effective and sustainable remediation.

Formation of metal carbonates

The integration of heavy metal ions into the evolving $CaCO_3$ structure is pivotal to the effectiveness of MICP. Heavy metal stabilisation is linked to the formation of metal carbonates, which render the metals less soluble and more stable. This process involves mechanisms such as isomorphic substitution of Ca^{2+}or incorporation into the crystal lattice interstices or defects (Tamayo-Figueroa *et al*., 2019). The efficiency of this incorporation depends on the ionic radius and charge of the heavy metal ions, which influence their ability to substitute for Ca^{2+}in the lattice. In addition, the presence of other ions can affect the formation and stability of metal carbonates, thereby influencing the overall stabilisation process. The saturation state of $CaCO_3$ in the environment also plays a role. High saturation levels promote nucleation and growth of $CaCO_3$ crystals, enhancing the entrapment of heavy metals. Conversely, low saturation levels may inhibit these processes, reducing immobilisation efficiency. The role of isomorphic substitution and incorporation into lattice defects is crucial but often complicated by the specific characteristics of each heavy metal ion (Eltarahony *et al*., 2021). For instance, the substitution of Ca^{2+} by comparable ionic radii such as Pb^{2+}, Cd^{2+}, or Zn^{2+} tends to be more efficient compared with metals with significantly different ionic radii or charges (e.g., Cr^{3+}, Cr^{6+}) (Mitra *et al*., 2022). In addition, the presence of competing ions such as Mg^{2+} and SO_4^{2-} can interfere with the formation of $CaCO_3$, necessitating a careful balance of ion concentrations in the treatment environment.

Microbial activity and metabolic processes

Microbial activity is a cornerstone of MICP, as the metabolic processes of microorganisms drive the precipitation of $CaCO_3$. Under adverse conditions, such as high heavy metal concentrations, microorganisms employ survival strategies such as the build-up and expulsion of calcium ions and the maintenance of a conducive

microenvironment. The metabolic activities that lead to the release of carbonate ions and subsequent $CaCO_3$ precipitation are influenced by factors such as exopolymers, biofilms, and dormant spores (Kim *et al*., 2021; Zhang *et al*., 2020). EPS produced by microbes bind heavy metals and serve as nucleation sites for $CaCO_3$ precipitation, enhancing the immobilisation process. Biofilms, which are complex microbial communities embedded in EPS, provide a protective environment for microbes and facilitate sustained metabolic activity and $CaCO_3$ production, even in the presence of toxic heavy metals (Crane *et al*., 2022; Zhao *et al*., 2020a). The protective nature of biofilms allows MICP microorganisms to survive and function under suboptimal conditions, which is advantageous in harsh environments. However, the heterogeneity of biofilms can result in uneven $CaCO_3$ precipitation and potential weak spots in the immobilisation matrix. Dormant spores add resilience to the microbial community, but their germination and subsequent metabolic activity are highly dependent on environmental triggers, which may not always be predictable or controllable. The complexity and variability of EPS composition can lead to inconsistent results, posing a challenge to the uniformity of $CaCO_3$ precipitation and the stability of the immobilisation matrix (Dong *et al*., 2023).

pH alterations and adenosine triphosphate (ATP) production

The interplay between ATP production and pH changes is critical in MICP. Proton flow through ATP synthase during cellular respiration causes pH shifts within the microenvironment, which are necessary for carbonate ion formation and $CaCO_3$ precipitation. These pH changes also significantly impact heavy metal stabilisation, as heavy metal ions co-precipitate with $CaCO_3$, rendering them insoluble (Zhang *et al*., 2022c). The production of ATP during cellular respiration is closely linked to pH modulation. Protons are shuttled during ATP synthesis, leading to pH changes that influence the solubility and speciation of heavy metals and carbonate ions (Jing *et al*., 2023; Qiao *et al*., 2021). These pH changes create a favourable environment for $CaCO_3$ precipitation and heavy metal stabilisation. Furthermore, the efficiency of ATP production and pH regulation is influenced by environmental factors such as temperature, oxygen availability, and nutrient concentration processes (Zhang *et al*., 2022a). Optimal conditions for microbial metabolism and ATP synthesis can enhance MICP processes, while suboptimal conditions can hinder these processes and reduce stabilisation efficiency. The correlation between ATP production and pH modulation is highly sensitive to environmental fluctuations. Temperature variations can significantly impact microbial respiration rates and, consequently, ATP production and pH changes. Oxygen availability is another critical factor; anaerobic conditions can limit the efficiency of aerobic microbes, leading to reduced ATP production and less effective pH modulation (Kang *et al*., 2022; Sharma *et al*., 2022b). Nutrient limitations can also hamper microbial growth and metabolic activity, necessitating the provision of a balanced nutrient supply to sustain effective MICP.

Conceptual evolution of the research field

Researchers can be enlightened about the historical perspective and the remarkable developmental milestones that have intricately shaped the trajectory of this field. Visual representation depicting the chronological evolution of MICP strategies for heavy metal removal, spanning from the 1970s to the 2020s, is shown in Figure 4. Understanding the historical perspective, key research

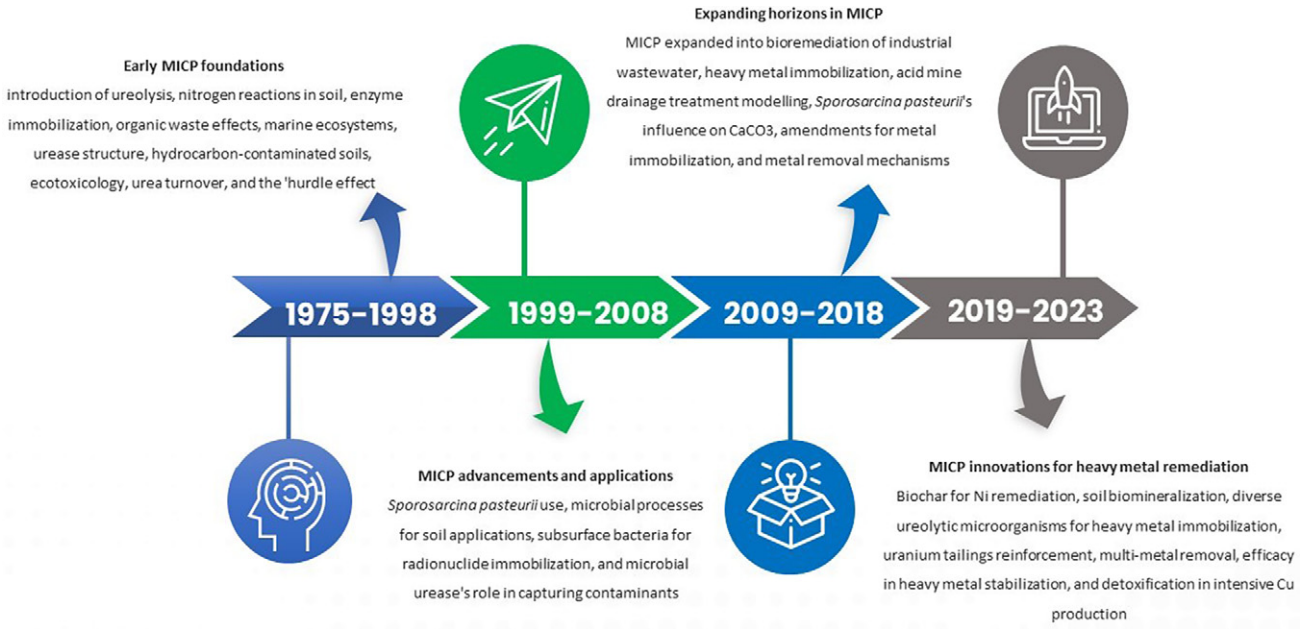

Figure 4. Chronological evolution of MICP strategies for heavy metal removal (the 1970s–2020s)

milestones, and hotspots of research themes and areas is vital for scholars in the field as it provides essential contex, and a roadmap of progress and identifies current areas of significance, enabling them to make informed contributions and advancements in their research

Early research (1975–1998) on MICP for heavy metal removal

Early research (1975–1998) laid the groundwork for understanding MICP's potential in heavy metal removal. Brown *et al*. (1975) introduced ureolysis, a key process in MICP, while Nannipieri *et al*. (1983, 1990) demonstrated ureolysis-driven ammonium release in soils. Bihari and Basu (1984) highlighted the industrial applications of immobilised urease, and Perucci (1990) and García *et al*. (1994) emphasised microbial influences on soil conditions and enzymatic activities. Goldstein (1994) extended the potential of MICP by uncovering microbial roles in marine carbon cycling. Benini *et al*. (1996) advanced the understanding of urease structure in *S. pasteurii*, crucial for ureolysis in MICP. Xu and Johnson (1997) accentuated the importance of microbial processes in soil nitrogen cycling in hydrocarbon-contaminated soils. Harder Nielsen *et al*. (1998) revealed rapid soil urea turnover's importance in nitrogen cycling. Ejechi and Akpomedaye (1998) showed how ureolytic microorganisms limited wood-rot fungi growth, indicating their ecological significance. Early studies provided foundational insights into the mechanisms of MICP, particularly ureolysis. However, the focus was predominantly on understanding basic microbial processes rather than direct applications for heavy metal removal. This period was crucial for setting the stage for future applied research but lacked targeted strategies for environmental remediation.

Advancements (1999–2008) in MICP for environmental remediation

Advancements (1999–2008) showcased MICP's efficacy in environmental remediation. Stocks-Fischer *et al*. (1999) and Fujita *et al*. (2000) placed the foundation for bacterial mineral precipitation and the ability to capture inorganic contaminants. Other important early studies underscored MICP's potential for environmental remediation, particularly in capturing inorganic contaminants (Bachmeier *et al*., 2002; Stuczynski *et al*., 2003; Warren *et al*., 2001). Hammes *et al*. (2003) explored industrial wastewater treatment through MICP, while Amos *et al*. (2004) and Rekha *et al*. (2005) demonstrated MICP's applicability in acid mine drainage and lake sediment remediation, respectively. Mitchell and Grant Ferris (2006) studied *S. pasteurii*'s impact on metal immobilisation, and Garau *et al*. (2007) investigated metal solubility reduction through amendments. Lee *et al*. (2008) studied metal removal in acid mine drainage, focusing on adsorption and showing sustained capacity of MICP over time. This period marked significant progress in applying MICP for environmental remediation. The research expanded beyond basic mechanisms to practical applications, demonstrating MICP's potential in diverse environmental contexts. However, these studies are still largely experimental and face challenges in scalability and field application.

Progress (2009–2018) in MICP for heavy metal immobilisation

Between 2009 and 2018, significant progress was made in the field. Researchers such as Sarda *et al*. (2009) showed MICP's potential in reducing water absorption in bricks, extending its use to heavy metal immobilisation. Li *et al*. (2010) demonstrated that ureolytic bacteria could immobilise Cd in contaminated soil with a 92% removal rate. Achal *et al*. (2011b, 2012b, 2012c, 2012a) successfully removed Cu from contaminated soil and extended the method to remediate Pb-, Sr-, and As-contaminated soil with up to 95% immobilisation efficiency. Li *et al*. (2013) explored biomineralisation of heavy metals (Ni, Cu, Pb, Co, Zn, Cd) by metal-resistant bacterial strains, achieving high removal rates (88%–99%). Achal *et al*. (2013) demonstrated the use of *Bacillus* sp. strain CS8 for the bioremediation of chromate (Cr (VI)) from chromium slag. Lauchnor *et al*. (2013) addressed Sr-contamination by way of ureolysis, suggesting a viable strategy for field-scale applications. Kang *et al*. (2014) investigated the biomineralisation of Cd, achieving a 99.95% removal rate. Kumari *et al*. (2014) explored MICP at low temperatures for Cd immobilisation in soil, showing a 90% removal rate. Kang *et al*. (2015) tackled Pb contamination, successfully immobilised Pb ions and achieved a 60% removal rate after 48 h of incubation. Kang *et al*. (2016) isolated bacteria from abandoned mines and used them for MICP to sequestrate Cu^{2+}, resulting in a 61.8% Cu immobilisation rate. Mwandira *et al*. (2017) addressed Pb-contaminated mine waste bioremediation using MICP, offering an eco-friendly method. Gui *et al*. (2018) reinforced fine-grained uranium tailings effectively through MICP, indicating its potential for tailings dam reinforcement. This period saw substantial advancements in applying MICP for heavy metal immobilisation. Studies demonstrated high removal rates and expanded the range of heavy metals addressed. However, while lab-scale experiments were successful, there were still significant challenges in translating these results to field-scale applications. Issues such as microbial survival in different environments, the consistency of ureolysis, and cost-effectiveness remained barriers.

Recent advances (2019–present) in MICP for heavy metal remediation

From 2019 onwards, MICP research for heavy metal removal has advanced significantly, demonstrating its potential in various environmental contexts. Zhang *et al*. (2019) introduced biochar to remediate Ni by way of MICP, finding that it inhibited calcite formation by *Bacillus cereus*, impacting Ni remediation. Khadim *et al*. (2019) used ureolytic bacteria from barn horse soil for Ni and Cd remediation, achieving up to 96% removal for Cd and 89% for Ni. Wang *et al*. (2020) isolated urease-producing bacteria from lettuce rhizosphere soil, effectively reducing Cd and Pb accumulation in lettuce. Peng *et al*. (2020) used a Cd-resistant ureolytic bacterium for MICP, achieving 99.50% Cd removal in

solution and 56.10% in soil. Qiao *et al*. (2021) demonstrated that *Sporosarcina* sp. could remove multiple heavy metals, with removal rates of 75%–98% within 2 hours. Bai *et al*. (2021) employed a halophilic ureolytic bacterium to remediate heavy metal-contaminated saline environments, achieving around 89% Pb removal under high salinity. He *et al*. (2022) used *Lysinibacillus fusiformis* for in situ biomineralisation of Cu–Ni tailings, significantly reducing heavy metal leaching. Disi *et al*. (2022) achieved 100% removal of Cr and zinc (Zn) using hydrocarbon-degrading ureolytic bacteria. Li *et al*. (2022b) reported high removal rates for Cd, Cu, and Pb with *S. pasteurii*. He *et al*. (2023) enhanced Pb immobilisation using ureolytic *Staphylococcus epidermidis* with polylysine, achieving a 92% immobilisation rate. Zeng *et al*. (2023) demonstrated 98.46% Cd immobilisation in sludge, with significant increases in urease metabolism genes. He *et al*. (2023) reported that adding calcium oxide (CaO) to MICP improved passivation rates for Cu, Ni, Pb, and Cr. Zhang *et al*. (2023) showed the potential of ureolytic microorganisms in detoxifying heavy metals from intensive Cu production, identifying mechanisms in areas with varying heavy metal content. Recent advancements in MICP research have shown its potential in various environmental contexts and achieved high removal rates for multiple heavy metals. Innovations such as the use of biochar, halophilic bacteria, and polylysine have enhanced the efficiency and applicability of MICP. However, challenges remain in scaling up these methods for practical, field-scale applications. Variability in microbial activity, environmental conditions, and cost-effectiveness are critical issues that need to be addressed to fully harness MICP's potential for heavy metal remediation.

Hotspots of research themes and areas

VOSviewer is an invaluable tool for identifying research hotspots and trends, helping researchers understand current dynamics and emerging areas of interest. Leading keywords based on frequency offer valuable insights into prominent research areas. The density visualisation of the author keyword using VOSviewer software displays the hotspot of the field as shown in Figure 5. 'MICP' underscores its central role, followed closely by 'urease' and 'heavy metals', highlighting the significance of this enzyme in the MICP process for heavy metal removal. Other dominant keywords include 'soil enzyme activities', 'bio-mineralisation', 'microbial communities', 'bacterial community', 'cadmium', '*Sporosarcina pasteurii*', and 'calcium carbonate', signifying specific elements and compounds that researchers are actively investigating. Keywords such as 'bio-cementation', 'enzymatic activities', 'rhizosphere microenvironment', 'microorganisms', 'constructed wetlands', and 'bio-remediation' emphasise the importance of these areas in current research. These keywords represent the focal

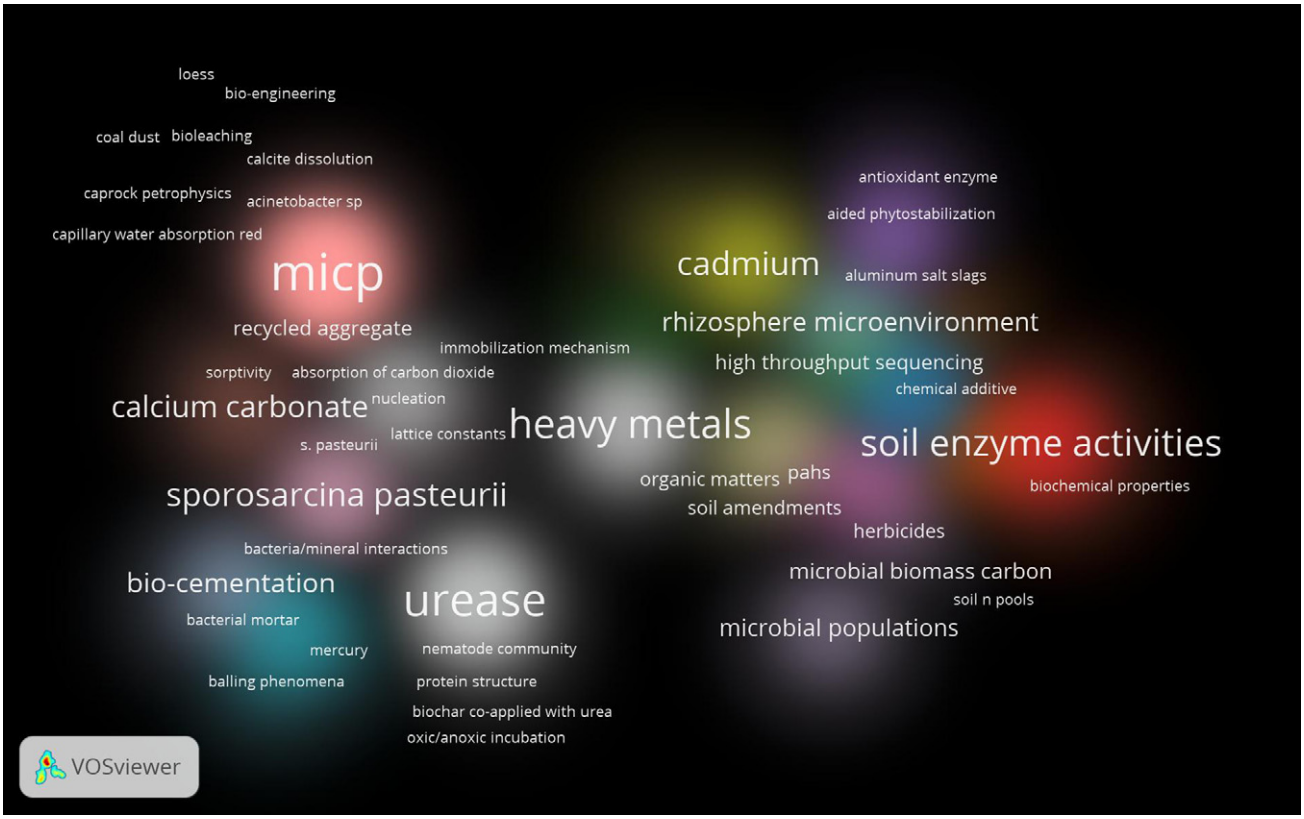

Figure 5. Density visualisation of author keyword hotspots using VOSviewer software

points of extensive academic literature and are instrumental in understanding the research landscape. In addition, less common but noteworthy keywords such as 'coal combustion fly ashes', 'environmental scanning electron microscope', 'ecophysiological index', and 'sustainable technology' offer a glimpse into the diverse and evolving research areas that researchers are exploring.

Data from the VOSviewer co-occurrence analysis of author keywords, illustrated in Figure S2, revealed key trends and focal points within the MICP field for heavy metal removal. The analysis shows 'Cd' as the most researched heavy metal, followed by 'Pb' and 'Cu', highlighting their significance as environmental pollutants and targets for MICP research. There is strong interest in terms such as 'biochar' and 'carbon', which enhance microbial activity and facilitate $CaCO_3$ precipitation. Enzymatic activities (e.g., 'catalase', 'glucosidase', 'acid phosphatase') and techniques (e.g., 'scanning electron microscopy', 'X-ray diffraction') are crucial in optimising MICP. The central role of microorganisms is emphasised with terms such as 'dehydrogenase', 'microbial community structure', and '*Sporosarcina pasteurii*'. Key terms such as 'absorption', 'accumulation', 'bioavailability', 'contaminated soil', and 'toxicity' underline the focus on heavy metal absorption and immobilisation. References to 'bioremediation', 'surface treatment', and 'cementitious material' highlight their importance, while terms such as 'biocementation', 'stabilisation', and 'carbon sequestration' suggest an emerging interest in broader environmental applications, such as soil strengthening and carbon dioxide capture. Different subgroups within these research themes are detailed in Figure S2.

Safety practices and sustainable approaches

MICP offers a promising and sustainable approach for heavy metal immobilisation, provided that safety practices are rigorously followed and continuously improved. To ensure the long-term safety and efficacy of MICP, further research is needed in several areas. Developing standardised protocols for monitoring and evaluating MICP performance in different contexts is essential. In addition, understanding the long-term durability of MICP-based structures and materials is crucial. While biomineralisation offers advantages, comprehending how these structures weather and degrade over time is vital for ensuring safety and sustainability. Understanding microbial processes, utilising waste materials, and incorporating machine learning are all crucial for enhancing the safety, efficiency, and environmental benefits of MICP. By focusing on these themes, MICP can be developed into a reliable and eco-friendly technique for environmental remediation.

Environmental safety

MICP holds the potential for reduced environmental impact compared with conventional techniques. Studies by Ivanov *et al*. (2019) and El Enshasy *et al*. (2020) highlight the importance of environmentally safe construction practices, noting that MICP avoids the release of harmful substances such as ammonia associated with traditional methods. This is critical, as ammonia can contribute to groundwater contamination and air pollution. In addition, Ivanov and Stabnikov (2020) demonstrate how MICP aligns with sustainable practices by promoting biomineralisation, which enhances the durability of structures while reducing the environmental footprint compared with traditional cement production. This biomineralisation process not only sequesters carbon dioxide but also produces less waste and consumes fewer natural resources.

Optimising safety and remediation performance

Safety practices can be improved by understanding the metabolic pathways of microorganisms used in MICP (Porter *et al*., 2021). This understanding is vital for controlling the process and preventing unintended environmental consequences. Ensuring the purity of input chemicals and by-products is also crucial, as contaminants could undermine the safety and effectiveness of MICP. Furthermore, scholars have showcased how machine learning can predict alternative stabilising materials that minimise environmental impact (Liu *et al*., 2022b; Raza and Khushnood, 2022; Zhang *et al*., 2022b). Machine learning can optimise the selection and combination of materials to enhance the efficacy and safety of MICP processes, thus making the approach more reliable and environmentally friendly. Studies have shown that MICP is a cleaner production approach for remediating contaminated soil and industrial materials, offering a sustainable alternative for construction materials (Mokhtar *et al*., 2021; Yu *et al*., 2021). This is particularly relevant for heavy metal immobilisation, as MICP can stabilise heavy metals in soils, preventing their leaching and reducing their bioavailability. In addition, researchers have investigated using waste materials such as kitchen waste in bioremediation processes (Sharma *et al*., 2022a). This approach not only provides a sustainable method for waste disposal but also adds value to waste materials, transforming them into useful inputs for MICP. Such practices highlight MICP's potential for sustainable building material production while addressing waste disposal concerns.

Sustainable soil improvement

Suriya and Sangeetha (2023) demonstrated how MICP can improve the erosive resistance of dispersive soil through the incorporation of jute fibres. This aligns with sustainable practices in geotechnical engineering by enhancing soil strength while mitigating erosion. The use of natural fibres such as jute not only reinforces soil but also promotes biodegradability and reduces reliance on synthetic materials. This approach exemplifies how MICP can be integrated with other sustainable practices to achieve multiple environmental benefits. Studies exploring industrial waste materials represent a breakthrough in heavy metal remediation methodologies. Bioaugmentation and biostimulation approaches utilise unconventional yet abundant resources such as waste materials (such as food scraps, agricultural residues, construction debris, or mining waste) to cultivate bacteria essential for MICP processes (Gomez *et al*., 2017; Raveh-Amit and Tsesarsky, 2020; Wang *et al*., 2014). This highlights the potential for safe and sustainable

MICP implementation. This not only reduces the need for expensive commercial bacterial cultures but also promotes sustainability by diverting waste materials from landfills and potentially lowering the environmental impact of MICP. Moreover, these waste-derived microbial communities may be more adaptable to various environmental conditions, harbouring a wider diversity of metal-resistant bacteria that can enhance heavy metal immobilisation through carbonate precipitation.

Challenges in MICP efficiency

High copper concentrations and acidic conditions

Cu is a crucial but toxic heavy metal, with soil levels often exceeding safety thresholds globally. This has led to bans in many countries due to heightened health and environmental risks. While MICP shows promise in controlled laboratory settings, its application in the field is challenging due to the need for controlled conditions (Chen and Achal, 2019). In addition, MICP is effective on numerous heavy metals including Cu. However, high Cu concentrations can inactivate the ureolytic bacteria crucial for the process (Xue *et al*., 2022). More so, social acceptance of using bacteria for bioremediation also varies by region. The US Environmental Protection Agency sets an action level of 1.3 mg/L for Cu in drinking water, while the World Health Organisation suggests a median value of 1.5 mg/L (Taylor *et al*., 2020; WHO, 2018).

Chen and Achal (2019) explored biostimulation to enhance MICP for Cu immobilisation in soil by spiking it with 100 mg/kg of Cu. Despite promising results, the study's short 1-month duration and controlled lab setting raise questions about long-term effectiveness and field applicability. The impact of biostimulation on Cu-resistant bacteria versus the general ureolytic population remains unclear, and the long-term effects on soil chemistry need further investigation. Sepúlveda *et al*. (2021) investigated *Staphylococcus equorum* and *S. pasteurii* for Cu removal using MICP, finding low removal rates (around 10%) insufficient for practical use. The formation of Cu–NH_3 complexes could prevent $CuCO_3$ precipitation, requiring additional measures to optimise Ca precipitation.

Heavy metal contamination from mine tailings poses serious environmental problems. Current treatment methods are often costly and ineffective. Yang *et al*. (2016) proposed using *Bacillus firmus* to remediate acidic Cu mine tailing soils, demonstrating that MICP could reduce heavy metal mobility. de Oliveira *et al*. (2021) examined MICP for treating Cu mine tailings, finding that *S. pasteurii*'s sensitivity to Cu (inhibited growth at 0.2–1 mM) limits its suitability. Anaerobic ureolytic bacteria may address oxygen transfer issues. Wang *et al*. (2023a) studied purified urease enzyme from jack bean seeds for Cu removal (5–50 mM). They found chitosan protects urease from Cu toxicity but increases NH_4^+ concentration, creating an alkaline environment where Cu forms complexes with NH_3, hindering immobilisation. Further research should focus on reducing Cu–ammonia complex formation and understanding carbonate-type effects on Cu immobilisation efficiency. The authors later compared urease from *S. pasteurii* and *Canavalia ensiformis* for Cu and Pb removal in water, achieving near 100% Pb removal but lower Cu removal (Wang *et al*., 2023b). They identified less stable carbonate precipitates (cotunnite and atacamite) under extreme conditions, potentially reducing remediation efficiency. High NH_4^+ concentrations in MICP can raise pH, promoting Cu–NH_3 complex formation that hinders Cu removal. Hu *et al*. (2024a) reported that *Brucella intermedia* effectively removes heavy metals, including Cu, from water. This bacterium has a complete urease gene cluster and efficiently uptakes urea, but the study's low Cu concentration (1 mmol/L) limits its generalisability. Further research with a broader range of Cu concentrations is needed. Hu *et al*. (2024b) also found *B. intermedia* highly resistant to Cu, with an IC50 value of 1.901 mmol/L. The bacteria effectively remove Cu through MICP, enhanced by Ca^{2+} co-precipitation with vaterite, facilitating Cu compound adhesion and precipitation. This study used a higher Cu concentration (1.6 mmol/L) compared with natural water levels (0.1–30 ppb), relevant for studying bioremediation in contaminated environments. Further research should focus on efficiency at varying contamination levels to develop practical strategies.

Heavy metal contamination in soil poses significant ecological and environmental threats, particularly in acidic soils prevalent in certain regions. These acidic conditions present unique challenges for remediation because they increase the mobility and bioavailability of heavy metals, making them more toxic (Yang *et al*., 2016). In addition, they limit the effectiveness of existing bioremediation methods that rely on carbonate-producing bacteria, as these bacteria often struggle in acidic environments (Saad *et al*., 2021). Therefore, novel bioremediation solutions are critically needed to effectively address heavy metal contamination in acidic soils. Hu *et al*. (2024c) explored the potential of *Lysinibacillus capsica* for bioremediating Cu in acidic soil (initial pH 5.16). The bacteria significantly increased the carbonate-bound state of the soil after 30 days, reducing metal mobility and bioavailability. In addition, *L. capsica* raised the soil pH during remediation, further reducing acidity and stabilising the heavy metals. However, large-scale field application effectiveness remains untested, requiring further research to evaluate long-term survival and effectiveness in acidic soils. Huang *et al*. (2024) studied a two-step MICP method for remediating high Cd concentration solutions. Direct exposure of *S. pasteurii* to Cd (10–40 mM) decreased urease activity, lowering Cd immobilisation efficiency. Simulations suggested nearly complete Cd removal when the carbonate to Cd concentration ratio was greater than 1:1. However, higher Cd concentrations limited carbonate production, resulting in incomplete Cd precipitation and decreased pH solution. Wang *et al*. (2023c) investigated a novel EK-PRB (Electrokinetic-Permeable Reactive Barrier) technology for removing Cu and Pb from soil. The bio-PRB (containing urease enzyme) in the EK reactor enhanced Cu and Pb removal. Acidic conditions near the anode promoted Cu and Pb desorption from the soil, allowing them to migrate towards the bio-PRB. There, they combined with CO_3^{2-} ions produced by

urease to form precipitates such as malachite (for Cu) and cerussite (for Pb), effectively removing them from the soil. However, acidic conditions could lead to competition, as some Cu and Pb might combine with CO_3^{2-} ions migrating towards the anode, reducing their removal by the bio-PRB itself.

Hu *et al*. (2023) addressed the challenge of acidic environments releasing Pb captured by biochar. They proposed using MICP to create a $CaCO_3$-surface barrier around the biochar after Pb capture. This barrier physically blocks contact between acids and Pb and chemically buffers against acidic attacks. The study demonstrated that MICP treatment significantly increased the stable fraction of Pb immobilised by biochar, with optimised conditions raising the stable Pb fraction from 4.8% to 92.5% compared with biochar alone. Further investigation is needed to validate its effectiveness in real-world soil settings. Xue *et al*. (2022) proposed a method to improve Cu immobilisation using MICP in acidic environments (pH < 4). They cultivated ureolytic microbes separately (without Cu) to produce NH_4^+ and OH^- ions and then added the culture solution to Cu-rich water. The OH^- ions reacted with CO_2 to form CO_3^{2-} for Cu immobilisation (as azurite or malachite, depending on final pH). This approach avoided exposing bacteria to high Cu concentrations, protecting their activity and promoting efficient Cu immobilisation even in Cu-rich environments. However, very high Cu concentrations (above 50 mM) could still affect the bacteria. Further research is needed to determine the ideal ratio of bacteria to Cu solution to avoid overly alkaline environments that hinder Cu immobilisation. In addition, the long-term stability of immobilised Cu and the potential for Cu release over time require further investigation.

Environmental impact

Despite the eco-friendliness of MICP as a biomediated, nature-based approach, certain environmental concerns necessitate further research and development to optimise its benefits. Key areas for optimisation include materials, processes, and performance of MICP applications. Jiang *et al*. (2022) highlighted that biostimulation could address the drawbacks of bioaugmentation, such as higher costs, unpredictable environmental risks, and labour-intensive procedures. In addition, MICP's engineering performance can be compromised by environmental factors such as wet–dry cycles, freeze–thaw cycles, and acid rain infiltration, which necessitates robust experimental approaches to characterise the durability of MICP-treated soils. Porter *et al*. (2021) reported that the ureolytic pathway, the most commonly used metabolic route for engineered MICP, has poor sustainability due to the high carbon footprint and energy demand of supplied urea, as well as the eutrophication potential of ammonium waste. They noted that MICP using ureolytic bacteria has the highest embodied energy (28.4 MJ), while MICP with carbonic anhydrase-producing bacteria has the lowest (12.9 MJ). The high embodied energy is predominantly due to laboratory-grade calcium chloride, which accounts for 44%–98% of the total energy. Wang *et al*. (2024) observed that the ammonia/ammonium by-products of ureolysis-driven MICP/ enzyme-induced carbonate precipitation EICP processes pose environmental risks such as eutrophication, oxygen depletion, and increased toxicity when released in excess. They emphasised optimising MICP/EICP solution dosages to avoid adverse effects on local flora and fauna, prevent root penetration issues, and manage residual chemicals such as unreacted $CaCl_2$ and urea that can alter soil salinity and affect plant growth.

Sun *et al*. (2024) evaluated the concentration of heavy metals and other pollutants in the waste slurry supernatant, comparing them to national standards to identify potential environmental hazards. They investigated the impact of waste slurry on soil chemistry and plant growth, finding that while MICP is effective for some heavy metals, it has limitations for others, such as arsenic. However, the study lacked a comprehensive analysis of the broader environmental impact of the entire MICP process, including air pollution, energy consumption, and mitigation strategies beyond MICP optimisation. Justo-Reinoso *et al*. (2023) conducted a life cycle assessment (LCA) of bacteria-based self-healing concretes (BBSHC), offering insights relevant to an EIA. The LCA compared the environmental impact of producing 1 m^3 of BBSHC with conventional concrete of similar strength and assessed the potential reduction in steel reinforcement needs. The study provided a basis for understanding BBSHC's environmental footprint by analysing factors such as energy consumption and material requirements. However, it did not explicitly state the environmental benefits or drawbacks compared with conventional concrete, nor did it provide a complete EIA. Future research should aim to bridge these gaps by developing more sustainable MICP methods, thoroughly evaluating long-term environmental impacts, and expanding the scope of EIAs to encompass the full life cycle and broader ecological consequences of MICP applications.

Cost considerations

Among the numerous studies on MICP for heavy metal abatement, only a few have explicitly projected MICP as a low-cost and eco-friendly method. However, the application of MICP in other fields, such as soil stabilisation, has a wealth of well-documented reports on the cost-effectiveness of the technique (Gowthaman *et al*., 2023; Omoregie *et al*., 2019b). This discrepancy highlights the need for more research focusing on the economic aspects of MICP for heavy metal immobilisation. For instance, Huang *et al*. (2024) proposed a two-step MICP method as a more cost-effective alternative. While the authors suggest a potentially more cost-effective method for Cd remediation, a comprehensive cost analysis is necessary to determine its true feasibility and widespread applicability. Xing *et al*. (2023) demonstrated the potential of MICP for Zn-contaminated soil remediation, but a crucial aspect, cost analysis, is missing. Without a comprehensive cost analysis considering scale-up, it is challenging to assess MICP's economic viability for extensive remediation projects. Furthermore, a comparison of MICP's costs with established Zn remediation techniques is lacking, which is essential to demonstrate its economic advantage.

Zeng *et al*. (2021) investigated MICP for remediating toxic metals in landfill leachate, mentioning its cost-effectiveness without providing a detailed cost breakdown. The economic viability for large-scale applications remains unclear without a thorough cost analysis considering the material, labour, equipment costs, and scalability challenges. Furthermore, the study does not account for all potential expenses, such as optimising urea dosage and recovering ammonium, which could impact its cost-effectiveness. Hu *et al*. (2021) explored MICP for removing calcium and contaminants from hypersaline-produced water, highlighting its potential for low cost due to the efficient use of urea and the possibility of ammonium recovery. However, a complete cost breakdown and comparison with other treatment methods are needed to assess MICP's relative cost-effectiveness accurately. Mwandira *et al*. (2017) investigated the use of *Pararhodobacter* sp. for Pb removal from contaminated sites, noting its effectiveness in lab studies. However, further investigation is needed for real-world applications, including a cost-effectiveness assessment.

Potential future directions for MICP to address challenges

Reducing copper toxicity

To improve MICP's effectiveness, scholars can explore genetic modification to develop bacteria with higher Cu tolerance. Utilising CRISPR-Cas9 or other gene-editing technologies (Hu *et al*., 2024a), researchers can insert/modify Cu-resistance genes in ureolytic bacteria. Alternatively, exposing bacteria to gradually increasing Cu concentrations can help select naturally occurring Cu-resistant strains. Investigating culture conditions that enhance ureolytic activity and CO_3 precipitation in the presence of Cu is crucial. Scholars can experiment with growth media formulations in MICP studies, such as adding chelating agents such as ethylenediaminetetraacetic acid or specific amino acids, and phosphate that bind Cu ions. Screening urease from extremophiles or Cu-tolerant plants can identify enzymes with high activity and stability in the presence of Cu ions. Investigating methods to immobilise urease on various substrates, such as silica gel, alginate beads, or polyurethane foams, can enhance their stability and reusability in MICP processes.

Acid-tolerant ureolytic bacteria

To enhance MICP in acidic soils, the identification or engineering of acid-tolerant ureolytic bacteria which can thrive in low-pH environments should be explored. Researchers in this area could also focus more on isolating new novel bacteria from underexplored acidic environments. Subsequent lab and field trials can assess the survival and efficacy of these engineered bacteria in acidic soils. Another approach to enhance MICP in acidic soils is soil pre-conditioning, which involves modifying soil properties before MICP application to create a more favourable environment for ureolytic bacteria and CO_3 precipitation. This could include the addition of buffering agents such as lime to raise soil pH to a level conducive to bacterial activity. Testing different application methods and monitoring soil pH changes over time can help optimise this pre-conditioning process. Comparing the effectiveness of two-stage approaches with traditional single-stage methods can provide insights into the optimal treatment strategy for acidic soils in terms of heavy metal immobilisation and soil pH stabilisation.

Long-term stability and monitoring

Investigating the long-term stability of MICP is crucial for assessing its effectiveness in immobilising heavy metals and preventing their release over time. Conducting field trials over several years can provide insights into the durability of the CO_3 precipitates formed and their resistance to environmental changes. Establishing methods to monitor changes in metal mobility, soil chemistry, and microbial communities over time can help identify potential issues early and guide adjustments to the treatment process. Continuous monitoring of soil parameters can provide valuable data on the long-term impact of MICP on the environment and help researchers refine their approach for optimal remediation outcomes. Field trials and environmental monitoring can provide valuable insights into the long-term effectiveness and environmental impact of MICP, ultimately enhancing its applicability as a sustainable remediation technique.

Minimising ammonia production

Mitigating the effects of NH_4^+ production in MICP is crucial for improving the efficiency and sustainability of the process. Future scholars can minimise ammonia production by focusing on manipulating bacterial urease genes to reduce the enzyme's activity without affecting its ureolytic function. Another avenue is metabolic engineering, which involves modifying bacterial metabolic pathways to divert the carbon and nitrogen fluxes towards carbonate precipitation instead of ammonia formation. This can be achieved by enhancing the activity of enzymes involved in CO_3 precipitation pathways while reducing those involved in ammonia production. Enhancing Cu-CO_3 precipitation is another key area for future research such as using additives (i.e. organic ligands or polymers). This can also prevent their dissolution in the presence of ammonia. In addition, optimising the concentrations of reactants involved in carbonate precipitation can promote the formation of stable Cu-CO_3 precipitates.

Enhancing cost consideration in MICP for heavy metal immobilisation

Addressing the limitation of cost consideration in MICP for heavy metal immobilisation is crucial for several reasons. First, understanding the economic feasibility of MICP is essential for its widespread adoption and application in remediation projects. Without a comprehensive cost analysis, decision-makers may be hesitant to invest in MICP, opting for more traditional but potentially less sustainable remediation methods. Second, cost consideration is vital for optimising MICP processes and making them more efficient and affordable. By identifying cost-effective strategies and materials, researchers can enhance the viability and scalability of MICP for heavy metal immobilisation. Future research should focus on conducting detailed cost analyses that include all relevant expenses, such as material costs, labour costs, equipment costs, and waste management costs. In

addition, comparisons with other remediation techniques should be made to demonstrate the economic advantages of MICP. Moreover, studies should explore innovative approaches to reduce costs, such as using alternative materials, optimising process parameters, and integrating MICP with other remediation technologies. By addressing these aspects, future research can provide valuable insights into the cost-effectiveness of MICP for heavy metal immobilisation and pave the way for its wider application in environmental remediation.

Leveraging machine learning for optimal performance

Future research should increasingly leverage machine learning to improve MICP processes for cost-effective heavy metal remediation. By analysing data from experiments and field trials, machine learning algorithms can identify patterns and relationships to optimise various aspects of MICP, including culture conditions, additive formulations, and long-term stability predictions. For example, machine learning can predict optimal culture conditions for ureolytic bacteria and identify bacteria with high heavy metal resistance. In addition, it can enhance additive usage to reduce heavy metal toxicity and enhance co-precipitation. Furthermore, machine learning can model the effects of different interventions, aiding in the design of efficient and sustainable remediation strategies. By optimising reagent usage, improving microbial scalability, and assessing environmental impacts, machine learning enhances the viability and affordability of MICP as a remediation method. Future research should prioritise the integration of machine learning techniques to advance the field of MICP and its applications in heavy metal remediation.

Incorporating comprehensive environmental impact assessment

To advance the field of MICP for heavy metal removal, it is crucial to incorporate comprehensive EIA. Thorough environmental risk assessments for bacterial strains used in MICP are vital, including their potential to disrupt native microbial communities, become invasive, or introduce antibiotic resistance. Enhancing bacterial specificity and developing control mechanisms to prevent accidental release or uncontrolled growth will mitigate risks. Implementing life cycle monitoring programmes to track the long-term effects of bacterial processes on soil and concrete is necessary. This includes monitoring potential degradation, chemical leaching, and impacts on structural integrity and soil health. Exploring novel or engineered bacterial strains that produce less harmful metabolites can improve both environmental and material outcomes. Developing sustainable methods for large-scale production of bacterial spores used in MICP is important. This could involve exploring alternative growth substrates, optimising culturing processes to reduce waste, and investigating renewable energy sources. Expanding LCA studies to cover the entire MICP process, including resource extraction, spore production, transportation, and waste disposal, will provide a comprehensive understanding of the environmental footprint and identify areas for improvement. Investigating potential air quality impacts, including emissions from bacterial growth processes or dust generation, is essential. Developing mitigation strategies to minimise negative effects on air quality and optimising MICP processes to reduce energy consumption is crucial. Future research should focus on understanding the speciation and bioavailability of heavy metals immobilised by MICP and their long-term stability and mobility under different environmental conditions. Figure 6 provides a concise

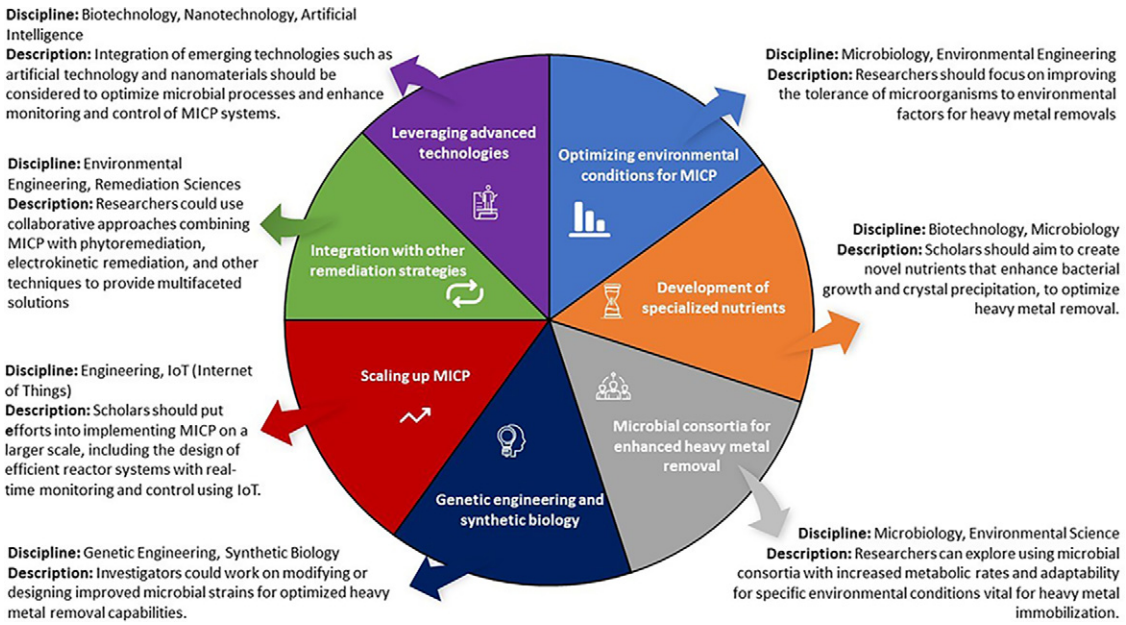

Figure 6. Pie chart representing the future directions of MICP applications for heavy metal removal

visual summary of the diverse and promising future directions, and opinions, reflecting the collective efforts aimed at promoting MICP as a sustainable and efficient solution for mitigating heavy metal contamination across various industries and disciplines.

Conclusion

This review highlights the substantial potential of MICP as a versatile bioremediation technology, extending beyond heavy metal removal to contribute to sustainable construction practices. The growing body of research underscores its effectiveness and adaptability. MICP's biogeochemical prowess, particularly through ureolysis, offers a scalable solution for the remediation of heavy metals, including Cu, Pb, Cd, Ni, and Zn. In addition, alternative pathways such as photosynthesis and nitrate reduction demonstrate its broad applicability in environmental clean-up. Looking ahead, continuous exploration and interdisciplinary collaboration are crucial to unlock MICP's full potential. Future advancements in nanomaterials and genetic engineering hold significant promise for further optimisation. A key area of focus is overcoming challenges associated with high Cu concentrations and highly acidic soils or tailings, which can inhibit the urease enzyme critical to MICP processes. Addressing these limitations and prioritising safety practices are essential for MICP to become a cornerstone of sustainable solutions for heavy metal contamination. Furthermore, enhancing cost considerations through detailed analyses and innovative approaches will be vital for the practical application and scalability of MICP. Leveraging machine learning to optimise MICP processes can also play a significant role in improving efficiency and cost-effectiveness. This review paves the way for researchers and industry experts to shape the future of MICP. Embracing collaboration and cutting-edge technologies can ensure MICP plays a critical role in achieving a cleaner and healthier environment, cementing its place as a key tool in environmental remediation and sustainable construction.

Authors contribution

A.I.O.: conceptualised and designed the review, conducted the literature review, and wrote and critically revised the manuscript. T.O. and D.E.L.O.: supervised the project, provided critical revisions, and ensured the overall integrity and accuracy of the review. H.F.B., K.M., O.O.O., D.J.F., and M.T.A.: assisted with the literature search, contributed to writing the discussion, and provided revisions based on peer review feedback. All authors read and commented on previous versions of the manuscript, and they collectively approved the final version.

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Conflict of interest

The authors declare no competing financial interests or personal relationships that could influence this study.

Data availability

The data supporting the findings of this study are available upon reasonable request. Interested parties may contact the corresponding author (T.O.) or the first author (A.I.O.) for access.

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