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Omoregie, AI, Ouahbi, T, Basri, HF, Ong, DEL, Muda, K, Ojuri, OO, Flores, DJ and Ammami, MT (2024) Heavy metal immobilisation with microbial-induced carbonate precipitation: a review. Geotechnical Research. pp. 1-25.

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1 **Heavy metal immobilisation with microbial-induced carbonate precipitation: A review**

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31 **ABSTRACT**

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

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47 ***Keywords:***

48 Biominalisation; Geochemical interaction; Heavy metal remediation; Microbial-induced carbonate
49 precipitation; Cost-effective remediation; UN SDG 6: Clean Water and Sanitation

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61 1. Introduction

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

70 The proportion of heavy metal pollution varies by industry, with mining contributing approximately 20-
71 40%, manufacturing 30-50%, and agriculture 10-20% (Su *et al.*, 2023). These estimates depend on industry type,
72 location, and regulatory frameworks. For example, mining in developing nations may have higher pollution levels
73 due to less stringent regulations, while agricultural pollution can vary with pesticide and fertiliser use (Mostafa *et*
74 *al.*, 2023; Vácha, 2021). Specific pollution sources include ore extraction and waste management in mining,
75 smelting and electroplating in manufacturing, and pesticides, fertilisers, sewage sludge, and livestock manure in
76 agriculture (Vácha, 2021; Wang *et al.*, 2021; Zhou *et al.*, 2022). While industrial and natural activities contribute
77 significantly to heavy metal pollution, it is critical to evaluate the effectiveness of current regulatory frameworks
78 and identify areas needing stricter enforcement or updated policies.

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

87

88 [INSERT FIGURE HERE]

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

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

99 Several techniques have been developed to address heavy metal pollution, including physicochemical
100 adsorption using biochar and natural zeolite ion exchangers (Bai *et al.*, 2023; Widiastuti *et al.*, 2011), and the
101 decomposition of heavy metal complexes through advanced oxidation processes and biogranulation (Basri *et al.*,
102 2023; Nidheesh *et al.*, 2022). However, these methods have significant limitations. Physicochemical adsorption
103 may be constrained by the low availability of functional groups in biochar, reducing ion exchange (Hama Aziz *et al.*,
104 2023). Advanced oxidation processes are costly and generate hazardous byproducts, requiring proper
105 treatment and disposal (Panwar and Pawar, 2022). Biogranulation is limited by the extended setup time and is
106 susceptible to environmental variations like temperature and pH (Omar *et al.*, 2023) The limitations of these
107 methods highlight the need for innovative approaches that enhance remediation efficiency while minimising
108 secondary environmental impacts.

109 Microbial-induced carbonate precipitation (MICP) is a biomineralisation process where microorganisms
110 facilitate the formation of calcium carbonate (CaCO₃) deposits. MICP holds significant promise in bioremediation
111 due to its ability to immobilise heavy metals and contaminants. The CaCO₃ crystals generated through MICP can
112 encase contaminants, reducing their bioavailability and leaching into the environment (Yang *et al.*, 2023). MICP
113 is effective for remediating soil and water contaminated by heavy metals (i.e., Pb, As, and Cd). Despite its
114 potential, the scalability and consistency of MICP applications in diverse environmental conditions require further
115 research. Researchers have explored the use of MICP for restoring concrete structures affected by corrosion and
116 chemical degradation (Achal *et al.*, 2011a; Joshi *et al.*, 2019). The potential of creating sustainable construction
117 materials, such as self-healing concrete and bio-bricks, is also under investigation (Farajnia *et al.*, 2022; Liu *et al.*,
118 2021). applications in soil biocementation, slope stabilisation, soil liquefaction, erosion control, and dust
119 mitigation offer eco-friendly alternatives to conventional methods, which often involve high-energy inputs and
120 synthetic chemicals (Gowthaman *et al.*, 2022; Mwandira *et al.*, 2019; Omoregie *et al.*, 2023; Sun *et al.*, 2021;

121 Wang *et al.*, 2022b). Compared to conventional grouting methods, microbial grouting using MICP techniques
122 offers advantages like reduced calcium consumption while maintaining the same compressive strength (Naeimi
123 and Haddad, 2020). These findings underscore the potential of eco-friendly alternatives in soil improvement.

124 MICP has wide-ranging applications in bioremediation and construction, making substantial contributions
125 to environmental and construction sustainability. Its efficacy has garnered significant attention, leading to a surge
126 in research and recognition as shown in **Table 1**. The number of publications on MICP increased from 2 in 1990
127 to 210 in 2023, with citations rising to 9,513 (see **Figure S1**). This growth reflects MICP's expanding scope,
128 potential to address environmental and construction challenges and significance in shaping innovative solutions.
129 Researchers and professionals increasingly recognise the value of MICP for heavy metal removal, a trend expected
130 to continue as MICP advances and proves its effectiveness in practical applications. MICP is particularly relevant
131 for heavy metal contamination.

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

140 [INSERT TABLE HERE]

141 **Table 1:** list of various heavy metals immobilised using MICP techniques.

142

143

144 **2. MICP Mechanism for Heavy Metal Removal**

145 2.1. Overview of MICP

146 MICP is a biogeochemical process where microorganisms produce minerals at low energy costs, offering
147 innovative possibilities in engineering applications (Murugan *et al.*, 2021). Among various types of
148 biomineralisation, MICP is classified as biologically induced mineralisation, where microorganisms alter the pH
149 of their surroundings, leading to carbonate precipitates (Bisht *et al.*, 2020; Power *et al.*, 2007; Zhi *et al.*, 2014).
150 While microorganisms do not directly control mineral deposit formation, they influence the process indirectly

151 through environmental pH changes. Understanding the interplay of physicochemical and biological factors is
152 crucial for optimising MICP efficiency, with bacterial cell concentration being a key factor (Murugan *et al.*, 2021).

153 In the MICP endeavours for heavy metal removal, the typical procedure encompasses several key steps. It
154 commences with the preparation of ureolytic bacterial cultures and the necessary sterilisation of reagents,
155 chemicals, and media. While conventional factors like biomass concentration/viability, pH, and urease activity
156 are monitored, precipitation tests are routinely executed to gauge the formation of carbonate precipitates and
157 appraise the resilience of bacterial cells against heavy metal ions present in the solution or medium (Xiao *et al.*,
158 2021). To showcase the transformative potential of soil biocementation, the MICP process is applied, occasionally
159 even in a liquid solution. Advanced analytical techniques are employed to scrutinise the MICP process and its
160 impact on the treated materials. Furthermore, the structural integrity and durability of materials treated through
161 MICP are evaluated using an unconfined compressive strength machine. Understanding MICP mechanisms is
162 essential for leveraging its potential in heavy metal immobilisation (**Figure 2**). This includes exploring the
163 preferred ureolysis pathway and alternative MICP pathways, highlighting their significant potential for heavy
164 metal remediation.

165

166 [INSERT FIGURE HERE]

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

172 2.2. Ureolysis pathway for MICP

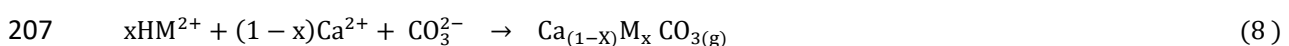
173 Ureolysis, or urea hydrolysis, is the most common pathway for MICP, primarily facilitated by urease-producing
174 bacteria such as *Sporosarcina pasteurii* and *Lysinibacillus sphaericus*. These bacteria hydrolyse urea using the
175 extracellular urease enzyme (Meier *et al.*, 2017). The hydrolysis process elevates the pH, promoting CaCO_3
176 precipitation from calcium and bicarbonate ions in solution. This pH elevation results from the consumption of
177 organic compounds, producing carbonate ions (CO_3^{2-}) and hydroxide ions (OH^-) as byproducts (Omoregie *et al.*,
178 2022). The resulting alkaline environment is essential for the subsequent precipitation of CaCO_3 .

179 Microorganisms play a dual role in biomineralisation. First, they create an environment conducive to
180 carbonate mineral formation by elevating the local pH through their metabolic processes (Xiao *et al.*, 2021).

181 Secondly, they produce extracellular polymeric substances (EPS), complex molecules serving as a matrix for Ca^{2+}
 182 and CO_3^{2-} ions to aggregate, forming initial mineral nuclei (Li *et al.*, 2017). EPS not only initiate mineral formation
 183 but also acts as an effective immobilising agent for heavy metals. Heavy metal ions bind to the EPS and integrate
 184 into the $CaCO_3$ structure, significantly reducing their mobility and bioavailability (Huang *et al.*, 2018).

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

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



208

209

210

211 [INSERT FIGURE HERE]

212 **Figure 3:** Schematic illustration showing the MICP mechanism for heavy metal immobilisation and co-
213 precipitation of CaCO₃.

214 Urease, a large enzyme complex with a molecular mass exceeding 500 kDa, contains two nickel (Ni) ions
215 vital for its catalytic function (Zhu *et al.*, 2021). This enzyme is essential for breaking down urea into carbonate
216 ions, which then react with calcium ions to form CaCO₃ precipitates, effectively trapping and immobilising heavy
217 metals. The Ni ions within the enzyme's active site are indispensable for catalytic activity, a prerequisite for
218 carbonate ion production. Carbonate ions subsequently react with calcium ions to generate CaCO₃ precipitates,
219 effectively trapping and immobilising heavy metals as demonstrated in **Figure 3**. The intricate molecular
220 mechanism governing urease catalysis is yet to be fully elucidated, but it has played a fundamental inspiration in
221 heavy metal immobilisation.

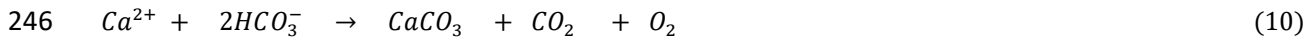
222 Ureolysis is preferred in MICP due to its simplicity, the abundance of urease-producing bacteria, and the
223 rapid pH elevation from liberated ammonia (Lauchnor *et al.*, 2015). These bacteria, such as *Sporosarcina*
224 *aquimarina*, *Sporosarcina pasteurii*, and *Exiguobacterium undae*, adapt well to various environmental conditions,
225 including alkaline pH and high calcium concentrations (Keykha *et al.*, 2019; Kumari *et al.*, 2014). This knowledge
226 forms a solid foundation for enhanced control and predictability within MICP processes. The accessibility and
227 affordability of urea also make MICP economically viable and scalable (Chen *et al.*, 2019, 2018; Omoregie *et al.*,
228 2019a). The extensive knowledge of urea hydrolysis and CaCO₃ precipitation by these bacteria provides valuable
229 insights for optimising the MICP process

230 2.3. Other MICP pathways

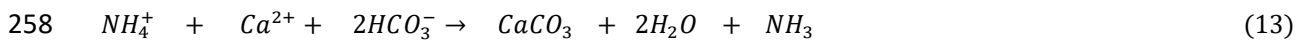
231 MICP can occur through various metabolic pathways (apart from ureolysis) through microorganisms, each
232 contributing to mineral precipitation. These distinct mechanisms include (i) photosynthesis, (ii) ammonification,
233 (iii) nitrate reduction (denitrification), (iv) sulfate reduction, and (v) iron reduction. Each of these pathways
234 contributes to mineral precipitation through different mechanisms.

235 Firstly, oxygenic photosynthesis unfolds as a captivating process. Specific microorganisms, exemplified
236 by cyanobacteria (i.e., *Synechococcus elongatus*), and algae (i.e., *Dinoflagellates*) harness the radiant energy of
237 sunlight to orchestrate a transformation (Bundeleva *et al.*, 2014; Frommlet *et al.*, 2015). This transformation sees
238 carbon dioxide (CO₂) and water metamorphose into organic matter while simultaneously liberating oxygen as a
239 noteworthy byproduct (Eqn. 9). The exchange of HCO₃⁻ or OH⁻ ions lead to an elevation in alkalinity across
240 microbial cells (Lamérand *et al.*, 2022). What makes this process especially intriguing is the ability of these

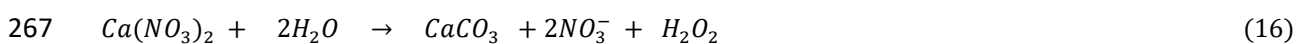
241 microorganisms to influence their immediate surroundings. Through the consumption of CO₂, they manipulate
 242 pH levels, setting the stage for the precipitation of CaCO₃ (Kawaguchi and Decho, 2002). This biomineralisation
 243 process is set into motion as conditions shift to favour CaCO₃ formation. As long there is increased alkalinity from
 244 the photosynthesis process facilitates the reaction between Ca²⁺ and HCO₃⁻, CaCO₃ minerals will form (Eqn. 10).



247 During ammonification microorganisms convert organic nitrogen-containing compounds, such as proteins
 248 and amino acids, into NH₃ (Eqn. 11). The generated NH₃ elevates the pH levels in the vicinity. The generated NH₃
 249 exhibits the remarkable capability to elevate the pH levels in the vicinity. Then, NH₃ react with HCO₃⁻ to form
 250 NH₄⁺ and CO₂ (Eqn. 12). The subsequent reactions with calcium ions lead to the precipitation of CaCO₃ (Eqn. 13),
 251 a key development in the MICP journey. While some bacteria, like *Virgibacillus marismortui*, acidify their
 252 environment (Zhao Z. et al., 2020), others, like *Brevibacillus laterosporus*, raise the pH through ammonification
 253 (Gunes and Balci, 2021). This increase in pH, observed in various species (i.e., *Rheinheimera texasensis*,
 254 *Paeniglutamicibacter kerguelensis*, *Ensifer adhaerens*, *Microbacterium testaceum*, and *Pseudomonas protegens*)
 255 (Hatayama and Saito, 2019) promotes the precipitation of CaCO₃, a key step in MICP.



259 Denitrifying bacteria contribute to MICP through a distinctive pathway. These bacteria use nitrate (NO₃⁻)
 260 as a terminal electron acceptor, and in the process, they generate nitric oxide (NO) (Eqn. 14). NO, in turn, can
 261 react with calcium ions, forming calcium nitrate (Ca(NO₃)₂) (Liu et al., 2022a). This metabolic feat leads to an
 262 intriguing pH elevation outcome. As the pH undergoes this shift, it lays the foundation for the precipitation of
 263 CaCO₃, an essential aspect of MICP. For instance, calcium nitrate is hydrolysed, leading to the precipitation of
 264 CaCO₃ and the release of nitric acid (Eqn. 15-16).



268 *Halomonas* sp and *Thauera* sp are reported microbial species known for their versatile metabolism and
269 ability to form biofilms (Chetty *et al.*, 2022). This process is not well understood, especially at alkaline pH levels.
270 Researchers observed a decrease in nitrate reduction rates from 0.72 mM/h at pH 9.5 to 0.17 mM/h at pH
271 exceeding 11, while the concentration of nitrite increased (Chetty *et al.*, 2022). The pH shift sets the stage for the
272 precipitation of CaCO₃, which plays a key role in MICP. The denitrification-driven pathway demonstrates the
273 adaptability of the process (Pham *et al.*, 2018).

274 Sulfate reduction is a pivotal process contributing to sulfide generation in environments with high sulfate
275 concentrations and low oxygen levels, both in natural and industrial contexts (Ren *et al.*, 2022). Certain sulfate-
276 reducing microorganisms (i.e., *Desulfovibrio* sp) induce MICP by producing sulfide ions (S²⁻). In this metabolic
277 pathway, microorganisms pivot towards sulfate (SO₄²⁻) as a terminal electron acceptor during anaerobic
278 respiration (Karnachuk *et al.*, 2021). Then, sulfate later converts into S²⁻ via a transformational phase (Eqn. 17).
279 These sulfide ions then react with Ca²⁺ in the environment, resulting in the formation of calcium sulfide (CaS)
280 (Chetty *et al.*, 2022; Gao *et al.*, 2023). Subsequently, this calcium sulfide can be oxidised, typically by atmospheric
281 oxygen or other oxidants, leading to the conversion of CaS into CaCO₃ and elemental sulfur (S) (Eqn. 18-19).



285 Iron reduction unfolds as a fascinating metabolic pathway where microorganisms channel the potential of
286 ferric iron (Fe³⁺) as an electron acceptor during anaerobic respiration. Iron-oxidising bacteria such as
287 *Sideroxydans* sp, *Gallionella* sp, and *Azoarcus* sp play a role in MICP by reducing (Fe³⁺) to ferric iron (Fe²⁺)
288 (Eqn. 20) (Levett *et al.*, 2020). The produced ferric iron interacts with HCO₃⁻ in the environment, forming ferric
289 hydroxide (Fe(OH)₃) and CO₂ (Eqn. 21) (Yang *et al.*, 2022). This ferric hydroxide subsequent transformation
290 leads to the precipitation of FeCO₃ and H₂O (Eqn. 22). The iron-mediated pathway adds another layer of
291 complexity to the MICP process (Ning *et al.*, 2022). Meanwhile, the release of CO₂ occurs during the initial
292 reaction. The precipitation of CaCO₃ is not explicitly shown in this pathway, as FeCO₃ is the primary product in
293 this case. Microbial iron reduction influences pH by balancing proton (H⁺) consumption during iron reduction
294 and (H⁺) generation from organic substrate oxidation (Li *et al.*, 2022a). In Fe³⁺ rich conditions, it increases pH,
295 converting dissolved CO₂ into bicarbonate. Beyond urea hydrolysis, MICP pathways can immobilise heavy metals

296 like Pb, Cd, and Cu by forming less soluble compounds. This adds complexity to MICP, which influences the pH
297 and contributes to FeCO₃ formation.



301

302

303 **3. Factors Influencing MICP Effectiveness**

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

308 3.1. Formation of Metal Carbonates

309 The integration of heavy metal ions into the evolving CaCO₃ structure is pivotal to the effectiveness of MICP.
310 Heavy metal stabilisation is linked to the formation of metal carbonates, which render the metals less soluble and
311 more stable. This process involves mechanisms such as isomorphous substitution of Ca²⁺ or incorporation into the
312 crystal lattice interstices or defects (Tamayo-Figueroa *et al.*, 2019). The efficiency of this incorporation depends
313 on the ionic radius and charge of the heavy metal ions, which influence their ability to substitute for Ca²⁺ in the
314 lattice. Additionally, the presence of other ions can affect the formation and stability of metal carbonates, thereby
315 influencing the overall stabilisation process. The saturation state of CaCO₃ in the environment also plays a role.
316 High saturation levels promote nucleation and growth of CaCO₃ crystals, enhancing the entrapment of heavy
317 metals. Conversely, low saturation levels may inhibit these processes, reducing immobilisation efficiency. The
318 role of isomorphous substitution and incorporation into lattice defects is crucial but often complicated by the
319 specific characteristics of each heavy metal ion (Eltarahony *et al.*, 2021). For instance, the substitution of Ca²⁺ by
320 comparable ionic radii such as Pb²⁺, Cd²⁺, or Zn²⁺ tends to be more efficient compared to metals with significantly
321 different ionic radii or charges (e.g., Cr³⁺, Cr⁶⁺) (Mitra *et al.*, 2022). Additionally, the presence of competing ions
322 such as Mg²⁺ and SO₄²⁻ can interfere with the formation of CaCO₃, necessitating a careful balance of ion
323 concentrations in the treatment environment.

324

325

326 3.2. Microbial Activity and Metabolic Processes

327 Microbial activity is a cornerstone of MICP, as the metabolic processes of microorganisms drive the precipitation
328 of CaCO₃. Under adverse conditions, such as high heavy metal concentrations, microorganisms employ survival
329 strategies like the buildup and expulsion of calcium ions and the maintenance of a conducive microenvironment.
330 The metabolic activities that lead to the release of carbonate ions and subsequent CaCO₃ precipitation are
331 influenced by factors such as exopolymers, biofilms, and dormant spores (Kim *et al.*, 2021; Zhang *et al.*, 2020).
332 EPS produced by microbes bind heavy metals and serve as nucleation sites for CaCO₃ precipitation, enhancing
333 the immobilisation process. Biofilms, which are complex microbial communities embedded in EPS, provide a
334 protective environment for microbes and facilitate sustained metabolic activity and CaCO₃ production, even in
335 the presence of toxic heavy metals (Crane *et al.*, 2022; Zhao *et al.*, 2020a). The protective nature of biofilms
336 allows MICP microorganisms to survive and function under suboptimal conditions, which is advantageous in
337 harsh environments. However, the heterogeneity of biofilms can result in uneven CaCO₃ precipitation and
338 potential weak spots in the immobilisation matrix. Dormant spores add resilience to the microbial community, but
339 their germination and subsequent metabolic activity are highly dependent on environmental triggers, which may
340 not always be predictable or controllable. The complexity and variability of EPS composition can lead to
341 inconsistent results, posing a challenge to the uniformity of CaCO₃ precipitation and the stability of the
342 immobilisation matrix (Dong *et al.*, 2023).

343 3.3. pH Alterations and Adenosine triphosphate (ATP) Production

344 The interplay between ATP production and pH changes is critical in MICP. Proton flow through ATP synthase
345 during cellular respiration causes pH shifts within the microenvironment, which are necessary for carbonate ion
346 formation and CaCO₃ precipitation. These pH changes also significantly impact heavy metal stabilisation, as
347 heavy metal ions co-precipitate with CaCO₃, rendering them insoluble (Zhang *et al.*, 2022c). The production of
348 ATP during cellular respiration is closely linked to pH modulation. Protons are shuttled during ATP synthesis,
349 leading to pH changes that influence the solubility and speciation of heavy metals and carbonate ions (Jing *et al.*,
350 2023; Qiao *et al.*, 2021). These pH changes create a favourable environment for CaCO₃ precipitation and heavy
351 metal stabilisation. Furthermore, the efficiency of ATP production and pH regulation is influenced by
352 environmental factors such as temperature, oxygen availability, and nutrient concentration processes (Zhang *et*
353 *al.*, 2022a). Optimal conditions for microbial metabolism and ATP synthesis can enhance MICP processes, while
354 suboptimal conditions can hinder these processes and reduce stabilisation efficiency. The correlation between ATP
355 production and pH modulation is highly sensitive to environmental fluctuations. Temperature variations can

356 significantly impact microbial respiration rates and, consequently, ATP production and pH changes. Oxygen
357 availability is another critical factor; anaerobic conditions can limit the efficiency of aerobic microbes, leading to
358 reduced ATP production and less effective pH modulation (Kang *et al.*, 2022; Sharma *et al.*, 2022b). Nutrient
359 limitations can also hamper microbial growth and metabolic activity, necessitating the provision of a balanced
360 nutrient supply to sustain effective MICP.

361

362

363 4. Conceptual Evolution of the Research Field

364 Researchers can be enlightened about the historical perspective and the remarkable developmental milestones that
365 have intricately shaped the trajectory of this field. Visual representation depicting the chronological evolution of
366 MICP strategies for heavy metal removal, spanning from the 1970s to the 2020s is shown in **Figure 4**.
367 Understanding the historical perspective, key research milestones and hotspots of research themes and areas is
368 vital for scholars in the field as it provides essential context, and a roadmap of progress, and identifies current
369 areas of significance, enabling them to make informed contributions and advancements in their research

370 [INSERT FIGURE HERE]

371 **Figure 4:** Chronological evolution of MICP strategies for heavy metal removal (the 1970s-2020s).

372

373 4.1. Early Research (1975-1998) on MICP for Heavy Metal Removal

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

386 was crucial for setting the stage for future applied research but lacked targeted strategies for environmental
387 remediation.

388 4.2. Advancements (1999-2008) in MICP for Environmental Remediation

389 Advancements (1999-2008) showcased MICP's efficacy in environmental remediation. Stocks-Fischer *et al.*,
390 (1999) and Fujita *et al.*, (Fujita *et al.*, 2000) placed the foundation for bacterial mineral precipitation and the ability
391 to capture inorganic contaminants. Other important early studies underscored MICP's potential for environmental
392 remediation, particularly in capturing inorganic contaminants (Bachmeier *et al.*, 2002; Stuczynski *et al.*, 2003;
393 Warren *et al.*, 2001). Hammes *et al.*, (2003), explored industrial wastewater treatment through MICP, while Amos
394 *et al.*, (2004) and Rekha *et al.*, (2005) demonstrated MICP's applicability in acid mine drainage and lake sediment
395 remediation, respectively. Mitchell and Ferris (2006) studied *Sporosarcina pasteurii's* impact on metal
396 immobilisation, and Garau *et al.*, (2007) investigated metal solubility reduction through amendments. Lee *et al.*,
397 (2008) studied metal removal in acid mine drainage, focusing on adsorption and showing sustained capacity of
398 MICP over time. This period marked significant progress in applying MICP for environmental remediation. The
399 research expanded beyond basic mechanisms to practical applications, demonstrating MICP's potential in diverse
400 environmental contexts. However, these studies are still largely experimental and face challenges in scalability
401 and field application.

402 4.3. Progress (2009-2018) in MICP for Heavy Metal Immobilisation

403 Between 2009 and 2018, significant progress was made in the field. Researchers like Sarda *et al.*, (2009) showed
404 MICP's potential in reducing water absorption in bricks, extending its use to heavy metal immobilisation. Li *et*
405 *al.*, (2010) demonstrated that ureolytic bacteria could immobilise Cd in contaminated soil with a 92% removal
406 rate. Achal *et al.*, (2011; 2012b; 2012c; 2012a) successfully removed Cu from contaminated soil and extended the
407 method to remediate Pb, Sr, and As-contaminated soil with up to 95% immobilisation efficiency. Li *et al.*, (2013)
408 explored biomineralisation of heavy metals (Ni, Cu, Pb, Co, Zn, Cd) by metal-resistant bacterial strains, achieving
409 high removal rates (88% to 99%). Achal *et al.*, (2013) demonstrated the use of *Bacillus* sp. strain CS8 for the
410 bioremediation of chromate (Cr (VI)) from chromium slag. Lauchnor *et al.*, (2013) addressed Sr-contamination
411 via ureolysis, suggesting a viable strategy for field-scale applications. Kang *et al.*, (2014) investigated the
412 biomineralisation of Cd, achieving a 99.95% removal rate. Kumari *et al.*, (2014) explored MICP at low
413 temperatures for Cd immobilisation in soil, showing a 90% removal rate. Kang *et al.*, (2015) tackled Pb
414 contamination, successfully immobilised Pb ions and achieved a 60% removal rate after 48 hours of incubation.
415 Kang *et al.*, (2016) isolated bacteria from abandoned mines and used them for MICP to sequester Cu²⁺, resulting

416 in a 61.8% Cu immobilisation rate. Mwandira *et al.*, (2017) addressed Pb-contaminated mine waste
417 bioremediation using MICP, offering an eco-friendly method. Gui *et al.*, (2018) reinforced fine-grained uranium
418 tailings effectively through MICP, indicating its potential for tailings dam reinforcement. This period saw
419 substantial advancements in applying MICP for heavy metal immobilisation. Studies demonstrated high removal
420 rates and expanded the range of heavy metals addressed. However, while lab-scale experiments were successful,
421 there were still significant challenges in translating these results to field-scale applications. Issues such as
422 microbial survival in different environments, the consistency of ureolysis, and cost-effectiveness remained
423 barriers.

424 4.4. Recent Advances (2019-Present) in MICP for Heavy Metal Remediation

425 From 2019 onwards, MICP research for heavy metal removal has advanced significantly, demonstrating its
426 potential in various environmental contexts. Zhang *et al.*, (2019) introduced biochar to remediate Ni via MICP,
427 finding that it inhibited calcite formation by *Bacillus cereus*, impacting Ni remediation. Khadim *et al.*, (2019)
428 used ureolytic bacteria from barn horse soil for Ni and Cd remediation, achieving up to 96% removal for Cd and
429 89% for Ni. Wang *et al.*, (2020) isolated urease-producing bacteria from lettuce rhizosphere soil, effectively
430 reducing Cd and Pb accumulation in lettuce. Peng *et al.*, (2020) used a Cd-resistant ureolytic bacterium for MICP,
431 achieving 99.50% Cd removal in solution and 56.10% in soil. Qiao *et al.*, (2021) demonstrated that *Sporosarcina*
432 sp. could remove multiple heavy metals, with removal rates of 75-98% within two hours. Bai *et al.*, (2021)
433 employed a halophilic ureolytic bacterium to remediate heavy metal-contaminated saline environments, achieving
434 around 89% Pb removal under high salinity. He *et al.*, (2022) used *Lysinibacillus fusiformis* for in-situ
435 biomineralisation of Cu-Ni-tailings, significantly reducing heavy metal leaching. Disi *et al.*, (2022) achieved
436 100% removal of Cr and zinc (Zn) using hydrocarbon-degrading ureolytic bacteria. Li *et al.*, (2022) reported high
437 removal rates for Cd, Cu, and Pb with *Sporosarcina pasteurii*. He *et al.*, (2023) enhanced Pb immobilisation using
438 ureolytic *Staphylococcus epidermidis* with poly-Lysine, achieving a 92% immobilisation rate. Zeng *et al.*, (2023)
439 demonstrated 98.46% Cd immobilisation in sludge, with significant increases in urease metabolism genes. He *et*
440 *al.*, (2023) reported that adding calcium oxide (CaO) to MICP improved passivation rates for Cu, Ni, Pb, and Cr.
441 Zhang *et al.*, (2023) showed the potential of ureolytic microorganisms in detoxifying heavy metals from intensive
442 Cu production, identifying mechanisms in areas with varying heavy metal content. Recent advancements in MICP
443 research have shown its potential in various environmental contexts and achieved high removal rates for multiple
444 heavy metals. Innovations such as the use of biochar, halophilic bacteria, and poly-lysine have enhanced the
445 efficiency and applicability of MICP. However, challenges remain in scaling up these methods for practical, field-

446 scale applications. Variability in microbial activity, environmental conditions, and cost-effectiveness are critical
447 issues that need to be addressed to fully harness MICP's potential for heavy metal remediation.

448 4.5. Hotspots of Research Themes and Areas

449 VOSviewer is an invaluable tool for identifying research hotspots and trends, helping researchers understand
450 current dynamics and emerging areas of interest. Leading keywords based on frequency offer valuable insights
451 into prominent research areas. The density visualisation of the author keyword using VOSviewer software displays
452 the hotspot of the field as shown in **Figure 5**. "MICP" underscores its central role, followed closely by "urease"
453 and "heavy metals", highlighting the significance of this enzyme in the MICP process for heavy metal removal.
454 Other dominant keywords include "soil enzyme activities", "bio-mineralisation", "microbial communities",
455 "bacterial community", "cadmium", "*Sporosarcina pasteurii*", and "calcium carbonate", signifying specific
456 elements and compounds that researchers are actively investigating. Keywords such as "bio-cementation",
457 "enzymatic activities", "rhizosphere microenvironment", "microorganisms", "constructed wetlands", and "bio-
458 remediation" emphasise the importance of these areas in current research. These keywords represent the focal
459 points of extensive academic literature and are instrumental in understanding the research landscape. Additionally,
460 less common but noteworthy keywords such as "coal combustion fly ashes", "environmental scanning electron
461 microscope", "ecophysiological index", and "sustainable technology" offer a glimpse into the diverse and
462 evolving research areas that researchers are exploring.

463

464 [INSERT FIGURE HERE]

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

466

467 Data from the VOSviewer co-occurrence analysis of author keywords, illustrated in Figure S2, revealed
468 key trends and focal points within the MICP field for heavy metal removal. The analysis shows "Cd" as the most
469 researched heavy metal, followed by "Pb" and "Cu", highlighting their significance as environmental pollutants
470 and targets for MICP research. There is strong interest in terms like "biochar" and "carbon", which enhance
471 microbial activity and facilitate CaCO₃ precipitation. Enzymatic activities (e.g., "catalase", "glucosidase", "acid
472 phosphatase") and techniques (e.g., "scanning electron microscopy", "X-ray diffraction") are crucial in optimising
473 MICP. The central role of microorganisms is emphasised with terms like "dehydrogenase", "microbial community
474 structure", and "*Sporosarcina pasteurii*". Key terms such as "absorption", "accumulation", "bioavailability",
475 "contaminated soil", and "toxicity" underline the focus on heavy metal absorption and immobilisation. References

476 to “bioremediation”, “surface treatment”, and “cementitious material” highlight their importance, while terms like
477 “biocementation”, “stabilisation”, and “carbon sequestration” suggest an emerging interest in broader
478 environmental applications, such as soil strengthening and carbon capture. Different subgroups within these
479 research themes are detailed in **Figure S2**.

480

481

482 **5. Safety Practices and Sustainable Approaches**

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

492 5.1. Environmental Safety

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

501 5.2. Optimising Safety and Remediation Performance

502 Safety practices can be improved by understanding the metabolic pathways of microorganisms used in MICP
503 (Porter *et al.*, 2021). This understanding is vital for controlling the process and preventing unintended
504 environmental consequences. Ensuring the purity of input chemicals and byproducts is also crucial, as
505 contaminants could undermine the safety and effectiveness of MICP. Furthermore, scholars have showcased how

506 machine learning can predict alternative stabilising materials that minimise environmental impact (Liu *et al.*,
507 2022b; Raza and Khushnood, 2022; Zhang *et al.*, 2022b). Machine learning can optimise the selection and
508 combination of materials to enhance the efficacy and safety of MICP processes, thus making the approach more
509 reliable and environmentally friendly. Studies have shown that MICP is a cleaner production approach for
510 remediating contaminated soil and industrial materials, offering a sustainable alternative for construction materials
511 (Mokhtar *et al.*, 2021; Yu *et al.*, 2021). This is particularly relevant for heavy metal immobilisation, as MICP can
512 stabilise heavy metals in soils, preventing their leaching and reducing their bioavailability. Additionally,
513 researchers have investigated using waste materials like kitchen waste in bioremediation processes (Sharma *et al.*,
514 2022a). This approach not only provides a sustainable method for waste disposal but also adds value to waste
515 materials, transforming them into useful inputs for MICP. Such practices highlight MICP's potential for
516 sustainable building material production while addressing waste disposal concerns.

517

518 5.3. Sustainable Soil Improvement

519 Suriya and Sangeetha, (2023) demonstrated how MICP can improve the erosive resistance of dispersive soil
520 through the incorporation of jute fibres. This aligns with sustainable practices in geotechnical engineering by
521 enhancing soil strength while mitigating erosion. The use of natural fibres like jute not only reinforces soil but
522 also promotes biodegradability and reduces reliance on synthetic materials. This approach exemplifies how MICP
523 can be integrated with other sustainable practices to achieve multiple environmental benefits. Studies exploring
524 industrial waste materials represent a breakthrough in heavy metal remediation methodologies Bioaugmentation
525 and biostimulation approaches utilise unconventional yet abundant resources like waste materials (such as food
526 scraps, agricultural residues, construction debris or mining waste) to cultivate bacteria essential for MICP
527 processes (Gomez *et al.*, 2017; Raveh-Amit and Tsesarsky, 2020; Wang *et al.*, 2014). This highlights the potential
528 for safe and sustainable MICP implementation. This not only reduces the need for expensive commercial bacterial
529 cultures but also promotes sustainability by diverting waste materials from landfills and potentially lowering the
530 environmental impact of MICP. Moreover, these waste-derived microbial communities may be more adaptable to
531 various environmental conditions, harbouring a wider diversity of metal-resistant bacteria that can enhance heavy
532 metal immobilisation through carbonate precipitation.

533

534

535

536 6. Challenges in MICP Efficiency

537 6.1. High Copper Concentrations and Acidic Conditions

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

546 Chen & Achal (2019) explored biostimulation to enhance MICP for Cu immobilisation in soil by spiking
547 it with 100 mg/kg of Cu. Despite promising results, the study's short one-month duration and controlled lab setting
548 raise questions about long-term effectiveness and field applicability. The impact of biostimulation on Cu-resistant
549 bacteria versus the general ureolytic population remains unclear, and the long-term effects on soil chemistry need
550 further investigation. Sepúlveda *et al.*, (2021) investigated *Staphylococcus equorum* and *Sporosarcina pasteurii*
551 for Cu removal using MICP, finding low removal rates (around 10%) insufficient for practical use. The formation
552 of Cu-NH₃ complexes could prevent CuCO₃ precipitation, requiring additional measures to optimise Ca
553 precipitation.

554 Heavy metal contamination from mine tailings poses serious environmental problems. Current treatment
555 methods are often costly and ineffective. Yang *et al.*, (2016) proposed using *Bacillus firmus* to remediate acidic
556 Cu mine tailing soils, demonstrating that MICP could reduce heavy metal mobility. Oliveira *et al.*, (2021)
557 examined MICP for treating Cu mine tailings, finding that *Sporosarcina pasteurii*'s sensitivity to Cu (inhibited
558 growth at 0.2-1 mM) limits its suitability. Anaerobic ureolytic bacteria may address oxygen transfer issues. L
559 Wang *et al.*, (2023) studied purified urease enzyme from Jack bean seeds for Cu removal (5 to 50 mM). They
560 found chitosan protects urease from Cu toxicity but increases NH₄⁺ concentration, creating an alkaline
561 environment where Cu forms complexes with NH₃, hindering immobilisation. Further research should focus on
562 reducing Cu-ammonia complex formation and understanding carbonate-type effects on Cu immobilisation
563 efficiency. The authors later compared urease from *S. pasteurii* and *Canavalia ensiformis* for Cu and Pb removal
564 in water, achieving near 100% Pb removal but lower Cu removal (Wang *et al.*, 2023a). They identified less stable
565 carbonate precipitates (cotunnite and atacamite) under extreme conditions, potentially reducing remediation

566 efficiency. High NH_4^+ concentrations in MICP can raise pH, promoting Cu- NH_3 complex formation that hinders
567 Cu removal. Hu *et al.*, (2024) reported that *Brucella intermedia* effectively removes heavy metals, including Cu,
568 from water. This bacterium has a complete urease gene cluster and efficiently uptakes urea, but the study's low
569 Cu concentration (1 mmol/L) limits its generalisability. Further research with a broader range of Cu concentrations
570 is needed. X Hu *et al.*, (2024) also found *B. intermedia* highly resistant to Cu, with an IC50 value of 1.901 mmol/L.
571 The bacteria effectively remove Cu through MICP, enhanced by Ca^{2+} co-precipitation with vaterite, facilitating
572 Cu compound adhesion and precipitation. This study used a higher Cu concentration (1.6 mmol/L) compared to
573 natural water levels (0.1 ppb to 30 ppb), relevant for studying bioremediation in contaminated environments.
574 Further research should focus on efficiency at varying contamination levels to develop practical strategies.

575 Heavy metal contamination in soil poses significant ecological and environmental threats, particularly in
576 acidic soils prevalent in certain regions. These acidic conditions present unique challenges for remediation because
577 they increase the mobility and bioavailability of heavy metals, making them more toxic (Yang *et al.*, 2016).
578 Additionally, they limit the effectiveness of existing bioremediation methods that rely on carbonate-producing
579 bacteria, as these bacteria often struggle in acidic environments (Saad *et al.*, 2021). Therefore, novel
580 bioremediation solutions are critically needed to effectively address heavy metal contamination in acidic soils.
581 Hu, *et al.*, (2024) explored the potential of *Lysinibacillus capsica* for bioremediating Cu in acidic soil (initial pH
582 5.16). The bacteria significantly increased the carbonate-bound state of the soil after 30 days, reducing metal
583 mobility and bioavailability. Additionally, *L. capsica* raised the soil pH during remediation, further reducing
584 acidity and stabilising the heavy metals. However, large-scale field application effectiveness remains untested,
585 requiring further research to evaluate long-term survival and effectiveness in acidic soils. Huang *et al.*, (2024)
586 studied a two-step MICP method for remediating high Cd concentration solutions. Direct exposure of
587 *Sporosarcina pasteurii* to Cd (10-40 mM) decreased urease activity, lowering Cd immobilisation efficiency.
588 Simulations suggested nearly complete Cd removal when the carbonate to Cd concentration ratio was greater than
589 1:1. However, higher Cd concentrations limited carbonate production, resulting in incomplete Cd precipitation
590 and decreased pH solution. Wang *et al.*, (2023) investigated a novel EK-PRB (Electrokinetic-Permeable Reactive
591 Barrier) technology for removing Cu and Pb from soil. The bio-PRB (containing urease enzyme) in the EK reactor
592 enhanced Cu and Pb removal. Acidic conditions near the anode promoted Cu and Pb desorption from the soil,
593 allowing them to migrate towards the bio-PRB. There, they combined with CO_3^{2-} ions produced by urease to form
594 precipitates like malachite (for Cu) and cerussite (for Pb), effectively removing them from the soil. However,

595 acidic conditions could lead to competition, as some Cu and Pb might combine with CO_3^{2-} ions migrating towards
596 the anode, reducing their removal by the bio-PRB itself.

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

611 6.2. Environmental Impact

612 Despite the eco-friendliness of MICP as a bio-mediated, nature-based approach, certain environmental concerns
613 necessitate further research and development to optimise its benefits. Key areas for optimisation include materials,
614 processes, and performance of MICP applications. Jiang *et al.*, (2022) highlighted that bio-stimulation could
615 address the drawbacks of bio-augmentation, such as higher costs, unpredictable environmental risks, and labour-
616 intensive procedures. Additionally, MICP's engineering performance can be compromised by environmental
617 factors like wet-dry cycles, freeze-thaw cycles, and acid rain infiltration, which necessitates robust experimental
618 approaches to characterise the durability of MICP-treated soils. Porter *et al.*, (2021) reported that the ureolytic
619 pathway, the most commonly used metabolic route for engineered MICP, has poor sustainability due to the high
620 carbon footprint and energy demand of supplied urea, as well as the eutrophication potential of ammonium waste.
621 They noted that MICP using ureolytic bacteria has the highest embodied energy (28.4 MJ), while MICP with
622 carbonic anhydrase-producing bacteria has the lowest (12.9 MJ). The high embodied energy is predominantly due
623 to laboratory-grade calcium chloride, which accounts for 44-98% of the total energy. Wang *et al.*, (2024) observed
624 that the ammonia/ammonium by-products of ureolysis-driven MICP/EICP processes pose environmental risks

625 such as eutrophication, oxygen depletion, and increased toxicity when released in excess. They emphasised
626 optimising MICP/EICP solution dosages to avoid adverse effects on local flora and fauna, prevent root penetration
627 issues, and manage residual chemicals like unreacted CaCl₂ and urea that can alter soil salinity and affect plant
628 growth.

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

644

645 6.3. Cost Considerations

646 Among the numerous studies on MICP for heavy metal abatement, only a few have explicitly projected MICP as
647 a low-cost and eco-friendly method. However, the application of MICP in other fields, such as soil stabilisation,
648 has a wealth of well-documented reports on the cost-effectiveness of the technique (Gowthaman *et al.*, 2023;
649 Omoregie *et al.*, 2019b). This discrepancy highlights the need for more research focusing on the economic aspects
650 of MICP for heavy metal immobilisation. For instance, Huang *et al.*, (2024) proposed a two-step MICP method
651 as a more cost-effective alternative. While the authors suggest a potentially more cost-effective method for Cd
652 remediation, a comprehensive cost analysis is necessary to determine its true feasibility and widespread
653 applicability. Xing *et al.*, (2023) demonstrated the potential of MICP for Zn-contaminated soil remediation, but a
654 crucial aspect, cost analysis, is missing. Without a comprehensive cost analysis considering scale-up, it is

655 challenging to assess MICP's economic viability for extensive remediation projects. Furthermore, a comparison
656 of MICP's costs with established Zn remediation techniques is lacking, which is essential to demonstrate its
657 economic advantage.

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

669

670

671 **7. Potential Future Directions for MICP to Address Challenges**

672 7.1. Reducing Copper Toxicity

673 To improve MICP's effectiveness, scholars can explore genetic modification to develop bacteria with higher Cu
674 tolerance. Utilising CRISPR-Cas9 or other gene-editing technologies (Hu *et al.*, 2024a), researchers can
675 insert/modify Cu-resistance genes in ureolytic bacteria. Alternatively, exposing bacteria to gradually increasing
676 Cu concentrations can help select naturally occurring Cu-resistant strains. Investigating culture conditions that
677 enhance ureolytic activity and CO₃ precipitation in the presence of Cu is crucial. Scholars can experiment with
678 growth media formulations in MICP studies, such as adding chelating agents like EDTA or specific amino acids,
679 and phosphate that bind Cu ions. Screening urease from extremophiles or Cu-tolerant plants can identify enzymes
680 with high activity and stability in the presence of Cu ions. Investigating methods to immobilise urease on various
681 substrates, like silica gel, alginate beads, or polyurethane foams, can enhance their stability and reusability in
682 MICP processes.

683

684 7.2. Acid-Tolerant Ureolytic Bacteria

685 To enhance MICP in acidic soils, the identification or engineering of acid-tolerant ureolytic bacteria which can
686 thrive in low-pH environments should be explored. Researchers in this area could also focus more on isolating
687 new novel bacteria from underexplored acidic environments. Subsequent lab and field trials can assess the survival
688 and efficacy of these engineered bacteria in acidic soils. Another approach to enhance MICP in acidic soils is soil
689 pre-conditioning, which involves modifying soil properties before MICP application to create a more favourable
690 environment for ureolytic bacteria and CO₃ precipitation. This could include the addition of buffering agents such
691 as lime to raise soil pH to a level conducive to bacterial activity. Testing different application methods and
692 monitoring soil pH changes over time can help optimise this pre-conditioning process. Comparing the
693 effectiveness of two-stage approaches with traditional single-stage methods can provide insights into the optimal
694 treatment strategy for acidic soils in terms of heavy metal immobilisation and soil pH stabilisation.

695 7.3. Long-term Stability and Monitoring

696 Investigating the long-term stability of MICP is crucial for assessing its effectiveness in immobilising heavy
697 metals and preventing their release over time. Conducting field trials over several years can provide insights into
698 the durability of the CO₃ precipitates formed and their resistance to environmental changes. Establishing methods
699 to monitor changes in metal mobility, soil chemistry, and microbial communities over time can help identify
700 potential issues early and guide adjustments to the treatment process. Continuous monitoring of soil parameters
701 can provide valuable data on the long-term impact of MICP on the environment and help researchers refine their
702 approach for optimal remediation outcomes. Field trials and environmental monitoring can provide valuable
703 insights into the long-term effectiveness and environmental impact of MICP, ultimately enhancing its applicability
704 as a sustainable remediation technique.

705 7.4. Minimising Ammonia Production

706 Mitigating the effects of NH₄⁺ production in MICP is crucial for improving the efficiency and sustainability of the
707 process. Future scholars can minimise ammonia production by focusing on manipulating bacterial urease genes
708 to reduce the enzyme's activity without affecting its ureolytic function. Another avenue is metabolic engineering,
709 which involves modifying bacterial metabolic pathways to divert the carbon and nitrogen fluxes towards carbonate
710 precipitation instead of ammonia formation. This can be achieved by enhancing the activity of enzymes involved
711 in CO₃ precipitation pathways while reducing those involved in ammonia production. Enhancing Cu-CO₃
712 precipitation is another key area for future research such as using additives (i.e., organic ligands or polymers).

713 This can also prevent their dissolution in the presence of ammonia. Additionally, optimising the concentrations of
714 reactants involved in carbonate precipitation can promote the formation of stable Cu-CO₃ precipitates.

715 7.5. Enhancing Cost Consideration in MICP for Heavy Metal Immobilisation

716 Addressing the limitation of cost consideration in MICP for heavy metal immobilisation is crucial for several
717 reasons. Firstly, understanding the economic feasibility of MICP is essential for its widespread adoption and
718 application in remediation projects. Without a comprehensive cost analysis, decision-makers may be hesitant to
719 invest in MICP, opting for more traditional but potentially less sustainable remediation methods. Secondly, cost
720 consideration is vital for optimising MICP processes and making them more efficient and affordable. By
721 identifying cost-effective strategies and materials, researchers can enhance the viability and scalability of MICP
722 for heavy metal immobilisation. Future research should focus on conducting detailed cost analyses that include
723 all relevant expenses, such as material costs, labour costs, equipment costs, and waste management costs.
724 Additionally, comparisons with other remediation techniques should be made to demonstrate the economic
725 advantages of MICP. Moreover, studies should explore innovative approaches to reduce costs, such as using
726 alternative materials, optimising process parameters, and integrating MICP with other remediation technologies.
727 By addressing these aspects, future research can provide valuable insights into the cost-effectiveness of MICP for
728 heavy metal immobilisation and pave the way for its wider application in environmental remediation.

729 7.6. Leveraging Machine Learning for Optimal Performance

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

741

742 7.7. Incorporating Comprehensive Environmental Impact Assessment

743 To advance the field of MICP for heavy metal removal, it is crucial to incorporate comprehensive EIA. Thorough
744 environmental risk assessments for bacterial strains used in MICP are vital, including their potential to disrupt
745 native microbial communities, become invasive, or introduce antibiotic resistance. Enhancing bacterial specificity
746 and developing control mechanisms to prevent accidental release or uncontrolled growth will mitigate risks.
747 Implementing life cycle monitoring programmes to track the long-term effects of bacterial processes on soil and
748 concrete is necessary. This includes monitoring potential degradation, chemical leaching, and impacts on
749 structural integrity and soil health. Exploring novel or engineered bacterial strains that produce less harmful
750 metabolites can improve both environmental and material outcomes. Developing sustainable methods for large-
751 scale production of bacterial spores used in MICP is important. This could involve exploring alternative growth
752 substrates, optimising culturing processes to reduce waste, and investigating renewable energy sources.
753 Expanding LCA studies to cover the entire MICP process, including resource extraction, spore production,
754 transportation, and waste disposal, will provide a comprehensive understanding of the environmental footprint
755 and identify areas for improvement. Investigating potential air quality impacts, including emissions from bacterial
756 growth processes or dust generation, is essential. Developing mitigation strategies to minimise negative effects
757 on air quality and optimising MICP processes to reduce energy consumption is crucial. Future research should
758 focus on understanding the speciation and bioavailability of heavy metals immobilised by MICP and their long-
759 term stability and mobility under different environmental conditions. **Figure 6 provides a concise visual summary
760 of the diverse and promising future directions, and opinions, reflecting the collective efforts aimed at promoting
761 MICP as a sustainable and efficient solution for mitigating heavy metal contamination across various industries
762 and disciplines.**

763 **[INSERT FIGURE HERE]**

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

765

766

767 **8. Conclusion**

768 This review highlights the substantial potential of MICP as a versatile bioremediation technology, extending
769 beyond heavy metal removal to contribute to sustainable construction practices. The growing body of research
770 underscores its effectiveness and adaptability. MICP's biogeochemical prowess, particularly through ureolysis,

771 offers a scalable solution for the remediation of heavy metals, including Cu, Pb, Cd, Ni, and Zn. Additionally,
772 alternative pathways like photosynthesis and nitrate reduction demonstrate its broad applicability in environmental
773 cleanup. Looking ahead, continuous exploration and interdisciplinary collaboration are crucial to unlock MICP's
774 full potential. Future advancements in nanomaterials and genetic engineering hold significant promise for further
775 optimisation. A key area of focus is overcoming challenges associated with high Cu concentrations and highly
776 acidic soils or tailings, which can inhibit the urease enzyme critical to MICP processes. Addressing these
777 limitations and prioritising safety practices are essential for MICP to become a cornerstone of sustainable solutions
778 for heavy metal contamination. Furthermore, enhancing cost considerations through detailed analyses and
779 innovative approaches will be vital for the practical application and scalability of MICP. Leveraging machine
780 learning to optimise MICP processes can also play a significant role in improving efficiency and cost-
781 effectiveness. This review paves the way for researchers and industry experts to shape the future of MICP.
782 Embracing collaboration and cutting-edge technologies can ensure MICP plays a critical role in achieving a
783 cleaner and healthier environment, cementing its place as a key tool in environmental remediation and sustainable
784 construction.

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1295 **List of Chemical Notations**

- 1296 1. $\text{CO}(\text{NH}_2)_2$ - Urea
- 1297 2. H_2O - Water
- 1298 3. NH_3 - Ammonia
- 1299 4. NH_2COOH - Carbamic acid
- 1300 5. H_2CO_3 - Carbonic acid
- 1301 6. H^+ - Hydrogen ion
- 1302 7. HCO_3^- - Bicarbonate ion
- 1303 8. OH^- - Hydroxide ion
- 1304 9. NH_4^+ - Ammonium ion
- 1305 10. CO_3^{2-} - Carbonate ion
- 1306 11. Ca^{2+} - Calcium ion
- 1307 12. CaCO_3 - Calcium carbonate
- 1308 13. HM^{2+} - Heavy metal ion (general term for divalent heavy metal ions)
- 1309 14. HMCO_3 - Heavy metal carbonate

- 1310 15. $\text{Ca}_{(1-x)}\text{M}_x\text{CO}_3$ - Mixed calcium-heavy metal carbonate (where X is the fraction of heavy metal
 1311 substitution)
 1312 16. CO_2 - Carbon dioxide
 1313 17. O_2 - Oxygen
 1314 18. NO_3^- - Nitrate ion
 1315 19. NO - Nitric oxide
 1316 20. $\text{Ca}(\text{NO}_3)_2$ - Calcium nitrate
 1317 21. H_2O_2 - Hydrogen peroxide
 1318 22. SO_4^{2-} - Sulfate ion
 1319 23. S^{2-} - Sulfide ion
 1320 24. CaS - Calcium sulfide
 1321 25. Fe^{3+} - Ferric ion
 1322 26. e^- - Electron
 1323 27. Fe^{2+} - Ferrous ion
 1324 28. $\text{Fe}(\text{OH})_3$ - Ferric hydroxide
 1325 29. FeCO_3 - Ferrous carbonate
 1326 30. CaO - Calcium Oxide

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1329 **List of Equations**

- 1330 1. $\text{CO}(\text{NH}_2)_2 + \text{H}_2\text{O} \rightarrow \text{NH}_3 + \text{NH}_2\text{COOH}$
 1331 2. $\text{NH}_2\text{COOH} + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 + \text{NH}_3$
 1332 3. $\text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^-$
 1333 4. $2\text{NH}_3 + 2\text{H}_2\text{O} \leftrightarrow 2\text{OH}^- + 2\text{NH}_4^+$
 1334 5. $\text{HCO}_3^- + \text{H}^+ + 2\text{OH}^- \rightarrow \text{CO}_3^{2-} + 2\text{H}_2\text{O}$
 1335 6. $\text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_{3(\text{g})}$
 1336 7. $\text{HM}^{2+} + \text{CO}_3^{2-} \rightarrow \text{HMCO}_{3(\text{g})}$
 1337 8. $x\text{HM}^{2+} + (1-x)\text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{Ca}_{(1-x)}\text{M}_x\text{CO}_{3(\text{g})}$
 1338 9. $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{Organic matter} +$

- 1339 10. $Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + O_2$
- 1340 11. *Protein or amino acid* $\rightarrow NH_3$
- 1341 12. $NH_3 + HCO_3^- \rightarrow NH_4^+ + CO_2$
- 1342 13. $NH_4^+ + Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + 2H_2O + NH_3$
- 1343 14. $NO_3^- \rightarrow NO$
- 1344 15. $Ca^{2+} + 2NO_3^- \rightarrow Ca(NO_3)_2$
- 1345 16. $Ca(NO_3)_2 + 2H_2O \rightarrow CaCO_3 + 2NO_3^- + H_2O_2$
- 1346 17. $SO_4^{2-} \rightarrow S^{2-}$
- 1347 18. $Ca^{2+} + S^{2-} \rightarrow CaS$
- 1348 19. $CaS + O_2 \rightarrow CaCO_3 + S$
- 1349 20. $Fe^{3+} + e^- \rightarrow Fe^{2+}$
- 1350 21. $Fe^{2+} + HCO_3^- \rightarrow Fe(OH)_3$
- 1351 22. $Fe(OH)_3 \rightarrow FeCO_3 + H_2O$

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1354 **List of Abbreviations**

- 1355 1. MICP: Microbial-Induced Carbonate Precipitation
- 1356 2. Hg: Mercury
- 1357 3. As: Arsenic
- 1358 4. Pb: Lead
- 1359 5. Cr: Chromium
- 1360 6. Cd: Cadmium
- 1361 7. Cu: Copper
- 1362 8. Ni: Nickel
- 1363 9. EPS: Extracellular Polymeric Substances
- 1364 10. ATP: Adenosine Triphosphate
- 1365 11. ATPase: Adenosine Triphosphatase
- 1366 12. *S. pasteurii*: Sporosarcina pasteurii
- 1367 13. SEM: Scanning Electron Microscopy
- 1368 14. XRD: X-ray Diffraction

- 1369 15. EK-PRB: Electrokinetic-Permeable Reactive Barrier
1370 16. BBSHC: Bacteria-Based Self-Healing Concretes
1371 17. EIA: Environmental Impact Assessment
1372 18. LCA: Life Cycle Assessment
1373 19. IC50: Half Maximal Inhibitory Concentration
1374 20. WHO: World Health Organization
1375 21. CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats
1376 22. EDTA: Ethylenediaminetetraacetic Acid
1377 23. kDa: Kilodalton

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1380 **Acknowledgement**

1381 The authors appreciate the University Technology of Sarawak and Universiti Teknologi Malaysia for facilitating
1382 access to essential databases and resources for obtaining bibliometric data.

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1385 **Authors Contribution**

1386 A.I.O.: Conceptualised and designed the review, conducted the literature review, and wrote and critically revised
1387 the manuscript. T.O. and D.E.L.O.: Supervised the project, provided critical revisions, and ensured the overall
1388 integrity and accuracy of the review. H.F.B., K.M., O.O.O., D.J.F., and T.A.: Assisted with the literature search,
1389 contributed to writing the discussion, and provided revisions based on peer review feedback. All authors read and
1390 commented on previous versions of the manuscript, and they collectively approved the final version.

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1393 **Funding Statement**

1394 This research was made possible through the generous support of the Normandy region in France, with funding
1395 provided under grant number R2020-RIN-0043.

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1397 **Conflict of Interest**

1398 The authors declare no competing financial interests or personal relationships that could influence this study.

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1400 **Data Availability**

1401 The data supporting the findings of this study are available upon reasonable request. Interested parties may contact
1402 the corresponding author (TO) or the first author (AIO) for access.

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