1   **Solidification of sand by Pb(II)-tolerant bacteria for capping mine waste to control**

2   **metallic dust: Case of the abandoned Kabwe Mine, Zambia**

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Abstract

Environmental impacts resulting from historic lead and zinc mining in Kabwe, Zambia affect human health due to the dust generated from the mine waste that contains lead, a known hazardous pollutant. We employed microbially induced calcium carbonate precipitation (MICP), an alternative capping method, to prevent dust generation and reduce the mobility of contaminants. Pb-resistant Oceanobacillus profundus KBZ 1-3 and O. profundus KBZ 2-5 isolated from Kabwe were used to biocement the sand that would act as a cover to prevent dust and water infiltration. Sand biocemented by KBZ 1-3 and KBZ 2-5 had maximum unconfined compressive strength values of 3.2 MPa and 5.5 MPa, respectively. Additionally, biocemented sand exhibited reduced water permeability values of 9.6×10^{-8} m/s and 8.9×10^{-8} m/s for O. profundus KBZ 1-3 and KBZ 2-5, respectively, which could potentially limit the entrance of water and oxygen into the dump, hence reducing the leaching of heavy metals. We propose that these isolates represent an option for bioremediating contaminated waste by preventing both metallic dust from becoming airborne and rainwater from infiltrating into the waste. O. profundus KBZ 1-3 and O. profundus KBZ 2-5 isolated form Kabwe represent a novel species that has, for the first time, been applied in a bioremediation study.

**Highlights**

- Mine waste dump causing chronic Pb poisoning to humans by inhaling airborne dust
- Proposed to use biomineralization to prevent the generation of dust
- Isolated indigenous biomineralization bacteria showing biocementation capability
- Capping mine waste by biomineralizing bacteria that reduce risk to human health
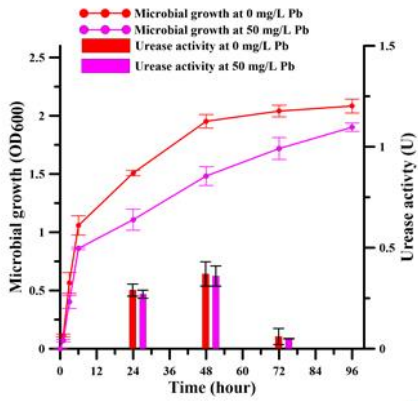- Effective, sustainable and novel approach to eliminate Pb poisoning
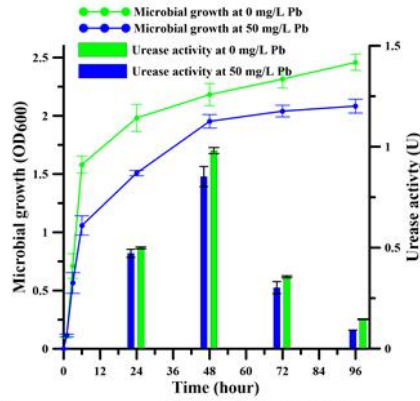
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Bacteria isolated from Pb-contaminated site
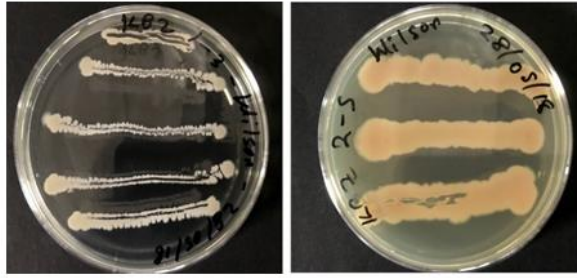
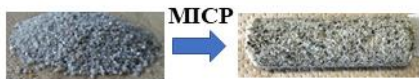

KBZ 1-3   **KBZ 2-5**

Microbial growth and urease activity in Pb (II)

O. profundus **KBZ 1-3**   *O. profundus* **KBZ 2-5**

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1 Introduction

Kabwe Mine was a lead and zinc mine that commenced its operations in 1902 until its closure in 1994. Apart from lead and zinc, it also produced silver, manganese, cadmium, vanadium, and titanium in smaller quantities (Mufinda, 2015). Due to the extraction of different minerals, several mineral processing techniques were used resulting in the production of, different types of mine waste dumps (Fig. 1a). Several studies have cited chronic Pb poisoning in humans, and contamination of soil, water, and sediments in the mine and the surrounding areas (Kribek et al., 2009; Tembo et al., 2006; Yabe et al., 2015; Yabe et al., 2018). The current blood levels of Pb in children exceeds 45 µg/dL, much higher than the recommended level of 5 µg/dL (Kosnett et al., 2007; Yabe et al., 2015). The predominant reason for this high Pb level in blood is the airborne Pb metallic dusts emanating from the mine waste dump. These dusts are blown by the prevailing winds into the residential areas, and due to their fine particulate nature, are either inhaled or ingested (Yabe et al., 2015). Leach plant residues and kiln slag, shown in Fig. 1b, are susceptible to wind and water erosion. These two types of wastes were selected for immobilization because they are deemed to be the most toxic and are distributed over the largest area onsite. In response to the concern of dust emanating from the mine wastes in Kabwe, remediation methods such as the revegetation of the waste dump by metallophytes was proposed and implemented, but subsequently failed because the plants failed to grow (Leteinturier et al., 2001). Additionally, mining waste has not been re-processed due to probably the cost of metal recovery (BMR Group PLC, 2019).

A promising technique to prevent metallic dust from becoming airborne in-situ is the immobilization of these wastes by microbially induced calcium carbonate precipitation (MICP) using ureolytic bacteria (Achal et al., 2013; Chen et al., 2017; Kim and Lee, 2018; Mwandira et al., 2017; Nam et al., 2016; Zhu et al., 2016). MICP involves the hydrolysis of urea into ammonium and carbarmate by urease catalysis which results in $CaCO_3$ formation in the presence

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67   of Ca^{2+} ions. The proposed use of MICP to cap mine wastes is expected to eliminate both dust

68   generation and water infiltration, restoring the contaminated site. Related studies have proposed

69   MICP for ground improvement (Salifu et al., 2016), coastal erosion control (Khan et al., 2015),

70   mine waste immobilization (Achal et al., 2013), self-healing concrete (Wiktor and Jonkers,

71   2011), and wastewater treatment (Torres-Aravena et al., 2018). Although many ureolytic bacteria

72   have been isolated, continued isolation and identification of more novel species, especially those

73   that are indigenous to the area, is indispensable. The present study focuses on (i) the isolation of

74   a Pb-resistant ureolytic bacteria from contaminated waste at Kabwe mine site; (ii) the

75   determination of the optimal Ca^{2+}/urea concentration, and (iii) the use of the bacteria to biocement

76   the sand. Such an investigation, involving the isolation and identification of effective

77   microorganisms for biotechnological applications, represents a sustainable approach to

78   remediation, eliminating the current environmental problems without significantly changing the

79   local ecological integrity. In this study, we introduced two new strains of ureolytic bacteria for

80   the MICP process: *Oceanobacillus profundus* KBZ 1-3 and *Oceanobacillus profundus* KBZ 2-5.

81

82   **2   Materials and methods**

83   **2.1   Soil sample collection**

84   Fig. 1a shows the locations of two soil sampling points from the abandoned Kabwe mine

85   site of Central Province, Zambia (15°27′–17°28′ S latitude and 23°06′–25°33′ E longitude). The

86   mine waste was exported from Zambia under approval No. RCT 7686229, and the import was

87   also permitted by Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan

88   under the approval No. 29-836. The samples were transported from the site to the Laboratory of

89   Biotechnology for Resource Engineering, Faculty of Engineering, Hokkaido University, Japan.

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2.2 Isolation and molecular identification of Pb-resistant bacteria

Bacteria were isolated by placing 1 g of soil in a 15 mL sterile centrifuge tube and adding 10 mL of sterile water, followed by vigorous shaking by hand. Samples were diluted 10- to 10,000-fold using sterile water and plated on NH4 YE agar medium (20 g/L yeast extract; 10 g/L di-ammonium sulfate $(NH_4)_2SO_4$; 0.13 M tris buffer (pH = 8.0); and 20 g/L agar amended with Pb(II)) to isolate Pb-resistant strains. The plates were incubated at 30 °C for 72 h. Urease activity was screened according to a previous study by Danjo and Kawasaki (2015). In brief, the isolated colonies were mixed with 20 mL of cresol red solution (25 g/L urea and 0.4 g/L cresol red) and left standing at 45 °C for 2 h. After 2 h, the samples that changed their color to purple were selected.

The Pb-resistant isolate was identified by 16S rRNA sequence analysis. DNA extracts were amplified using two sets of primers targeting the 16S rRNA region specific for almost all bacterial 16S sequences: primers F9 (5'- GAGTTTGATCCTGGCTCAG -3') and R1451 (5'- AAGGAGGTGATCCAGCC -3'). The PCR amplification cycle consisted of an initial denaturation step of 5 min at 94 °C, followed by 25 cycles of 1 min at 94 °C, 2 min at 60 °C, and 1 min at 72 °C and a final extension step of 30 min at 72 °C. The amplicons were separated by gel electrophoresis and the resulting DNA bands were extracted and purified using the FastGene™ PCR extraction Kit following the manufacturer's instructions (Nippon Genetics Co. Ltd, Tokyo, Japan). The extracted DNA was sent to Eurofins Genomics laboratory (Eurofins Genomics, Tokyo, Japan) for DNA sequencing. Subsequent phylogenetic analysis was conducted by TechnoSuruga Laboratory (TechnoSuruga Laboratory Company Ltd, Tokyo Japan), which used the BLAST algorithm to find related sequences in the GeneBank Database, DNA Data Bank of Japan and the European Molecular Biology Laboratory.

2.3 Effect of Pb on microbial growth and urease activity of isolates

Microbial growth and urease activity were measured according to the procedures reported in a previous study (Mwandira et al., 2017). Microbial growth was measured by UV–vis spectrophotometry (V-730, Jasco International Co., Ltd., Tokyo, Japan) that recorded optical density (OD) readings at 600 nm for 96 h in the absence and presence of 50 mg/L Pb(II). Experiments were conducted in triplicate.

2.4 Determination of the optimal Ca^{2+}/urea concentration

Bioprecipitation experiments were carried out to determine the optimal Ca^{2+}/urea concentrations. The bacterial isolate was precultured for 24 h in 5 mL NH4 YE medium, then 1 mL of preculture was inoculated into 100 mL of NH4 YE medium to grow the main culture at 30 °C for 24 h with continuous aeration at 160 rpm. The bacterial suspension was then added to different equimolar concentrations of $CaCl_2$ and urea (0.1 M, 0.3 M, 0.5 M, 0.75 M, and 1.0 M). The mixtures were subsequently incubated for 24 h at 30 °C with shaking (160 rpm) and then centrifuged (15,000 rpm for 5 min) to collect the precipitate. The precipitate was weighed and then analyzed by XRD. Precipitation experiments were conducted in triplicate.

2.5 Syringe biocementation test

The Mizunami sand used in the experiments is uniformly graded with a median particle size of 1.2 mm (Fig. S1). Mizunami sand was used to represent sand that can be obtained locally near the contaminated site. The sand was sterilized, and hand packed into a 35-mL syringe (mean diameter, $D_{50} = 2.5$ cm and height, h = 7 cm), followed by the gentle injection of bacteria and solidification solution as illustrated in Fig. S2 (Supplementary material). Initially bacteria suspension was injected and allowed to stand in the column for 2 h and thereafter solidification solution was injected. This was repeated every 24 h for a period of 14 days. Additionally, two

sets of biocementation experiments were conducted using conditions designed to mimic the possible conditions for in-situ injection of treatment solutions. In the first set of experiments, 2 mL of cementation solution was left above the surface of the sand to mimic saturated conditions; this procedure is called immersed method. The second set of experiments was conducted by sequentially adding solution as the solution was drained; thus, this procedure is called the flow-through method. Control tests were also conducted following the same procedures but without the addition of bacterial cells.

Unconfined compressive strength (UCS) of the cemented samples was measured using a needle penetration device (SH-70, Maruto Testing Machine Company, Tokyo, Japan) to determine the strength of biocemented sand prepared by immersed and flow-through methods.

The $CaCO_3$ contents of the cemented samples were determined by the calcimetric method, which uses 3M HCl acid and standard grade $CaCO_3$ (Hukue et al., 2001). In brief, 1.5 g solidified sand and 15 ml HCl in plastic vials were placed in a reaction vessel which was then closed, tightened, zeroed, and sealed with O-rings. The vessel was then shaken to allow HCl to react with sample, producing CO_2 gas. A digital manometer measured and recorded the CO_2 gas pressure readings. Same procedure was used to generate a calibration curve with known amounts of $CaCO_3$, which was used to quantify the readings from the specimen. The control and top center part of biocemented specimens treated by immersed and flow-through methods were tested.

2.6   Hydraulic conductivity

Hydraulic conductivity was assessed by the falling head method using a DIK 4000 system (Daiki Rika Kogyo Co., Ltd., Saitama, Japan). The samples were saturated with water and placed in a desiccator for 48 h before the measurement of hydraulic conductivity. Hydraulic conductivity was determined in control and biocemented specimens treated by both immersed and flow through methods.

2.7 SEM and XRD analysis

The microstructure of fractions of the biocemented samples was examined by scanning electron microscopy (SEM) (Miniscope TM3000, Hitachi, Tokyo, Japan). Additionally, X-ray diffraction (XRD) analysis (MiniFlex™, Rigaku Co., Ltd., Tokyo, Japan) was conducted using Ni-filtered Cu 1.5406 Å radiation to determine the mineral phases of both the control and biocemented sand. Scans were recorded from 5° to 80° of 2θ at a rate of 20°/min.

3 Results and discussion

3.1 Isolation of ureolytic bacteria

Ureolytic bacteria were isolated based on the color change of the cresol red solution after urea hydrolysis. Colonies that changed the color of the solution from yellow to purple were selected. Color change is observed due to urea hydrolysis that causes the pH of the medium to rise. Of the thirty-five isolates from Kabwe, only four isolates, identified as *Oceanobacillus profundus* KBZ 1-3, *Psychrobacillus* sp. KBZ 2-2, *Oceanobacillus profundus* KBZ 2-3, and *O. profundus* KBZ 2-5 were found to produce urease and tolerated Pb, and were screened. *O. profundus* KBZ 1-3 and *O. profundus* KBZ 2-5 were selected for subsequent experiments because they were Pb-resistant and capable of biocementation. Supplementary Fig. S3 shows the neighbor-joining phylogenetic tree of *O. profundus* KBZ 1-3 and *O. profundus* KBZ 2-5, which were isolated from the leach plant residue mine waste and near the wastewater pond, respectively. Both *O. profundus* 1-3 and *O. profundus* KBZ 2-5 are gram-positive, motile, aerobic, rod-shaped (0.2-0.4 μm and 0.5-0.6 μm respectively) and are classified as biosafety level 1 bacteria. The genus *Oceanobacillus* has been previously isolated from wastewater (Nam et al., 2008), Korean food (Whon et al., 2010), deep sea sediment core samples (Yu et al., 2014) and human gut (Lagier et al., 2015). To the best of our knowledge, there are no reports indicating their potential application in biotechnology, bioremediation or biosorption. Therefore, *O. profundus* KBZ 1-3

190   and *O. profundus* KBZ 2-5 isolated from Kabwe waste samples represent a novel *Oceanobacillus*

191   species that is being applied for the first time in a bioremediation study.

192

193   **3.2   Effect of Pb on microbial growth and urease activity of isolates**

194   Since the isolates are intended to be used in a heavily Pb-contaminated environment, the bacteria

195   were tested for the effect of Pb (II) in aqueous solution. Fig. 2 shows the effect of Pb on both

196   microbial growth and urease activity of *O. profundus* KBZ 1-3 and *O. profundus* KBZ 2-5. Both

197   bacteria displayed similar growth patterns, i.e., increased growth in lead-free media and a slight

198   growth retardation in the presence of 50 mg/L lead (Fig. 2). Therefore, the effects of lead on

199   microbial growth were minimal, likely because these bacteria were isolated from a lead-

200   contaminated site with bioavailable concentration of Pb (II) of 7.8 mg/L in leach plant residues

201   whereas kiln slag had 5.40 mg/L bioavailable lead concentration. The bioavailable fraction

202   determines the potential harm of a contaminant on the receptor (Ng et al., 2015). Similar results

203   have been reported from previous research where growth retardation was exhibited by a

204   halotolerant bacteria in the presence of lead isolated from an abandoned mine in South Korea

205   (Kang et al., 2015).

206   The effect of lead on urease activity was studied because it is crucial in MICP-mediated

207   bioremediation for the abandoned Kabwe mine site. As shown in Fig. 2, the urease activity *O.*

208   *profundus* KBZ 2-5 is higher than that of *O. profundus* KBZ 1-3; both bacteria expressed the

209   highest urease activity after 48 h incubation with only appreciable levels at 24 h and 72 h. Only

210   *O. profundus* KBZ 2-5 maintained the enzyme activity until 96 h. The urease activities of both

211   isolates were not significantly affected by lead, probably because they were isolated from a lead-

212   contaminated site. Higher urease activity is very important in MICP-mediated processes because

213   it has a significant impact on the rate of carbonate production that consequently precipitates out

214   as $CaCO_3$. The results clearly show increased growth and urease activity by *O. profundus* KBZ

215   1-3 and *O. profundus* KBZ 2-5 in the absence and presence of lead. Overall, both bacteria are

216   suitable for the biocementation of mine waste contaminated with Pb because they are Pb-tolerant,

217   with high growth and urease activities.

218

219   **3.3   Determination of the optimal Ca^{2+}/urea concentration**

220   Calcium and urea are the two most important ingredients for carrying out the MICP process.

221   The urease enzyme produced from the bacteria hydrolyzes urea ($CO(NH_2)_2$) to ammonium

222   (NH_4^+) and carbonate (CO_3^{2-}), which leads to the precipitation of $CaCO_3$ in the presence of

223   calcium ions (Ca^{2+}). Therefore, the tolerance to and the optimal concentrations of calcium and

224   urea may vary from one bacterial species to another, requiring the determination of optimal

225   conditions for *O. profundus* KBZ 1-3 and *O. profundus* KBZ 2-5. Fig. 3 shows the amount of

226   precipitate formed by both bacteria when the concentration ratio of calcium:urea = 1:1; equimolar

227   concentrations were used because they are more effective, according to a previous study (Soga

228   and Qabany, 2013). As shown in Fig. 3, increasing the equimolar calcium and urea concentrations

229   also increased the amount of precipitate. In this study, we used the equimolar concentration of

230   0.5 M for calcium and urea. Previous studies have indicated that a low equimolar concentration

231   in the solution should be used to ensure a uniform consistency of $CaCO_3$ precipitation (Mujah et

232   al., 2017). Since a solution with low concentration may produce a more uniform precipitation

233   pattern, even though higher concentrations produce higher amounts of $CaCO_3$ precipitate, a lower

234   concentration was selected. In this study, both *O. profundus* KBZ 1-3 and *O. profundus* KBZ 2-

235   5 precipitated spherical calcite crystals (Supplementary Fig. S4). Calcite is the preferred form of

236    $CaCO_3$ for biocementation because it is the most stable, compared to the other polymorphic forms

237   such as aragonite and vaterite (Boulos et al., 2014).

238   The two isolates precipitated $CaCO_3$, which can be used as an inert covering for mine

239   wastes. Capping is advantageous as a treatment technology because it is a permanent remedy that

240 can also eliminate dust, thus addressing chronic risks of Pb poisoning to humans and other

241 ecological receptors (Bellenfant et al., 2013; Johnson et al., 1992; Lottermoser, 2011).

242

243 **3.4   Strength, SEM and XRD analyses of solidified sand**

244   UCS was measured to characterize the strength of cemented sand. Fig. 4 shows the

245 appearance of the control and biocemented sand, while Table 1 shows the corresponding

246 estimated values of UCS at the top, middle, and bottom parts of the specimens.

247   The control specimen registered no strength while sand biocemented by *O. profundus*

248 KBZ 1-3 under immersed and flow-through conditions had maximum estimated UCS values of

249 3.2 MPa and 2.0 MPa, respectively. The strength of sand biocemented by *O. profundus* KBZ 2-

250 5 under immersed and flow-through conditions were 5.5 MPa and 3.5 MPa, respectively. Sand

251 biocemented by *O. profundus* KBZ 2-5 was stronger probably because of higher urease activity

252 (Fig. 2) and the greater amount of precipitate formed compared to *O. profundus* KBZ 1-3 (Fig.

253 3). Since the difference in UCS is marginal, both bacteria may be useful for solidification.

254 Additionally, both immersed and flow-through cementation methods provided more strength.

255 Therefore, both types of injection methods can be used for solidifying sand. The results imply

256 that the increased strength of biocemented sand has the potential to prevent the airborne transport

257 of metallic dust by prevailing winds and to reduce infiltration; these benefits are similar to those

258 of conventional cement, used worldwide for capping mining waste (Batchelor, 2006; Sobiecka,

259 2013).

260   To further confirm the role of MICP, biocemented samples were examined by SEM and

261 XRD. Fig. 5 shows typical SEM images and the corresponding XRD patterns of the control (a)

262 and biocemented sand (b, c). The control samples present a typical morphology of sand and

263 appear as discrete particles, while the biocemented samples show prominent crystalline deposits

264 on the surface and between sand particles. The SEM micrographs verify the effectiveness of the

265 so-called bridging phenomenon mediated by MICP, i.e. the deposited $CaCO_3$ forms bridges

266 between particles as part of the binding process (Mujah et al., 2017; Ng et al., 2012;

267 Rowshanbakht et al., 2016).

268 The XRD analysis reveals that the control specimens are composed of only quartz, while

269 the biocemented sand includes calcite. Calcite is formed due to the ureolysis and subsequent

270 precipitation of $CaCO_3$. The XRD results allowed us to conclude that MICP plays an important

271 role in the solidification of sand.

272 The major pathway for Pb to gain entry into the blood of humans and animals around the Kabwe

273 mine site is through inhalation and injection of dust particles emanating from the abandoned mine

274 wastes dumps, since the prevailing winds blow mostly from east (the mine site) to west (toward

275 a large residential area) (Tembo et al., 2006; Yabe et al., 2011, 2013, 2015, 2018). Immobilizing

276 the sand via MICP aggregates the sandy material (Fig. 4), making it less susceptible to being

277 blown by wind. This significantly curtails the Pb exposure pathway to humans and animals in

278 and around the mine site. MICP using indigenous bacteria can be immediate and easily

279 implemented because all the required materials such as sand, indigenous bacteria, nutrients,

280 calcium source, and urea are locally available. Some researchers have proposed the use of

281 alternative locally available nutrients and calcium sources such as lactose mother liquor (Achal

282 et al., 2009) and eggshells (Choi et al., 2016), which also demonstrates the flexibility of the

283 process. In a similar way, locally available resources required for capping will be utilized

284 including the indigenous bacteria making the bioremediation process cheap, sustainable, and less

285 likely to change the integrity of the local biodiversity.

286

287 **3.5 CaCO$_3$ content of biocemented sand**

288 To elucidate the strength of the biocement, the $CaCO_3$ content precipitated between sand grains

289 of specimens was evaluated. Only the top parts of the control and biocemented sand were

13

290   evaluated. The control contained no $CaCO_3$. On the other hand, sand biocemented by immersed

291   and flow methods using *O. profundus* KBZ 1-3 had 6.5±0.10 % and 3.0±0.20 % $CaCO_3$,

292   respectively, while sand biocemented by *O. profundus* KBZ 2-5 by immersed and flow methods

293   had 10.0±0.20 % and 8.0±0.20 % $CaCO_3$, respectively. $CaCO_3$ content is one of the most

294   important engineering factors in MICP-mediated processes. Its relationship with UCS is shown

295   in Fig. 6. As seen in the results, the control contained no $CaCO_3$ and hence had no strength. The

296   UCS of biocemented sand increased with $CaCO_3$ content (indicated by the pink and green

297   circles), which suggests that $CaCO_3$ plays a significant role in the strength of sand, which has

298   also been elucidated by previous studies (Amarakoon and Kawasaki, 2018). Furthermore, more

299   precipitation of $CaCO_3$ occurred in immersed method probably due to accumulation of reactants

300   and bacteria when the syringe is closed. The findings are in agreement with results reported by

301   Keykha et al., (2019) when they solidified soil and maintained immersed conditions at all times

302   and achieved higher UCS. Similarly, Gomez et al., (2018) reported that with the largest calcite

303   contents were observed near the injection which had higher UCS when they conducted

304   biostimulation and concluded that reductions in calcite content from the top were due to solution

305   mixing and/or urea hydrolysis. In both treatment methods, the mid-top area has higher UCS

306   because the reactants first contact the mid-top when injected where they are spent, depleted and

307   less effective hence the lower UCS in middle and bottom area. The difference in UCS between

308   the treatment methods lies in the contact time of the reactants in the column. In the immersed

309   method, the reactants have more contact time during immersed compared to flow through method

310   where reactants flow through the column in a shorter time hence the higher UCS in the immersed

311   method compared to flow through method.

312

313   **3.6   Hydraulic conductivity**

314   Hydraulic conductivity is a measure of how easily water can pass through a material. During

315   immobilization, reduced hydraulic conductivity is desired because it reduces the ability of water

to contact contaminants, and, therefore, reduces contaminant leaching rates. Hydraulic conductivity tests were performed on the sand before and after MICP treatment. Before treatment, the hydraulic conductivity was 1.4×10^{-3} m/s. Biocemented sand treated by the immersed and flow-through methods using *O. profundus* KBZ 1-3 reduced the water permeability of sand to 9.6×10^{-8} m/s and 2.4×10^{-7} m/s, respectively. Similarly, *O. profundus* KBZ 2-5 reduced the water permeability of sand to 8.9×10^{-8} m/s and 2.5×10^{-7} m/s when treated by immersed and flow-through methods, respectively. In all the cases, the hydraulic conductivity improved by more than three orders of magnitude for both immersed and flow-through methods. The reduced hydraulic conductivity achieved in this study has the potential to limit the entrance of water and oxygen into the dump, and hence reduce the leaching of heavy metals. This reduction in permeability is consistent with results of previous studies (Achal et al., 2013, Eryürük et al., 2015).

Other studies have proposed vegetation cover (Chehregani et al., 2009, Leteinturier et al., 2001) and synthetic cover (Fourie et al., 2010; Mazzieri et al., 2013) to cap mine wastes. Vegetation cover is desirable because like MICP, it reduces surface erosion and a large proportion of percolating water is lost to the atmosphere through transpiration, reducing the concentrations of soluble heavy metals entering watercourses. However, this method would be difficult to implement in Kabwe, because vegetation growth is not possible at the site due to lack of nutrients and high levels of toxic trace elements at the site (Leteinturier et al., 2001). On the other hand, synthetic covers are uneconomical and expensive, especially compared to the MICP technique. Due to its originality and sustainability, MICP has recently gained much attention from researchers around the world as a replacement for conventional concrete (Seifan et al., 2016). Conventional physicochemical methods have already been tested to clean the environment. However, most of these methods are costly, perform sub-optimally, and produce secondary sludge, making the cleanup process expensive and unsustainable, requiring large inputs of energy and large quantities of chemical reagents (Jena and Dey, 2016).

4 Conclusions

The abandoned lead and zinc mine wastes in Kabwe mine continue to pose a serious threat to the quality of human health, water, and soil. We have shown in this study that microbially induced calcium carbonate precipitation (MICP) mediated by indigenous ureolytic bacteria *Oceanobacillus profundus* KBZ 1-3 and KBZ 2-5 can be used to solidify sand, thus preventing dust formation and water infiltration. Both bacteria were able to tolerate Pb and mediate the formation of $CaCO_3$ bioprecipitates, which was confirmed to be calcite by XRD analysis. The biocemented sand achieved maximum unconfined compressive strength values of 3.2 MPa and 5.5 MPa, which are useful enough to prevent Pb dust particles from being blown away by prevailing winds and to prevent water erosion. Combined with reduced hydraulic conductivity of 9.6×10^{-8} m/s and 8.9×10^{-8} m/s mediated by *Oceanobacillus profundus* KBZ 1-3 and KBZ 2-5, respectively, the process is expected to retard heavy metal leaching due to the lack of oxygen and water resulting from reduced infiltration. In a future study, we intend to implement this laboratory-proven procedure in-situ to determine the durability of biocemented materials under field conditions.

Declarations of interest: none

Appendix A. Supplementary material

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508   **Fig. 1.** (a) Location of soil sampling sites and the different mine wastes at the abandoned mine,

509   Kabwe, Zambia (b) Appearance of leach plant residues and kiln slag.

510   **Fig. 2.** Microbial growth and urease activity of (a) *O. profundus* KBZ 1-3, and (b) *O. profundus*

511   KBZ 2-5. Error bars indicate standard deviations of three independent replicates. (U=µmol urea

512   hydrolyzed/min)

513   **Fig. 3.** Weight of $CaCO_3$ bioprecipitated by *O. profundus* KBZ 1-3 and *O. profundus* KBZ 2-5

514   at different equimolar concentrations of calcium and urea. Error bars indicate the standard
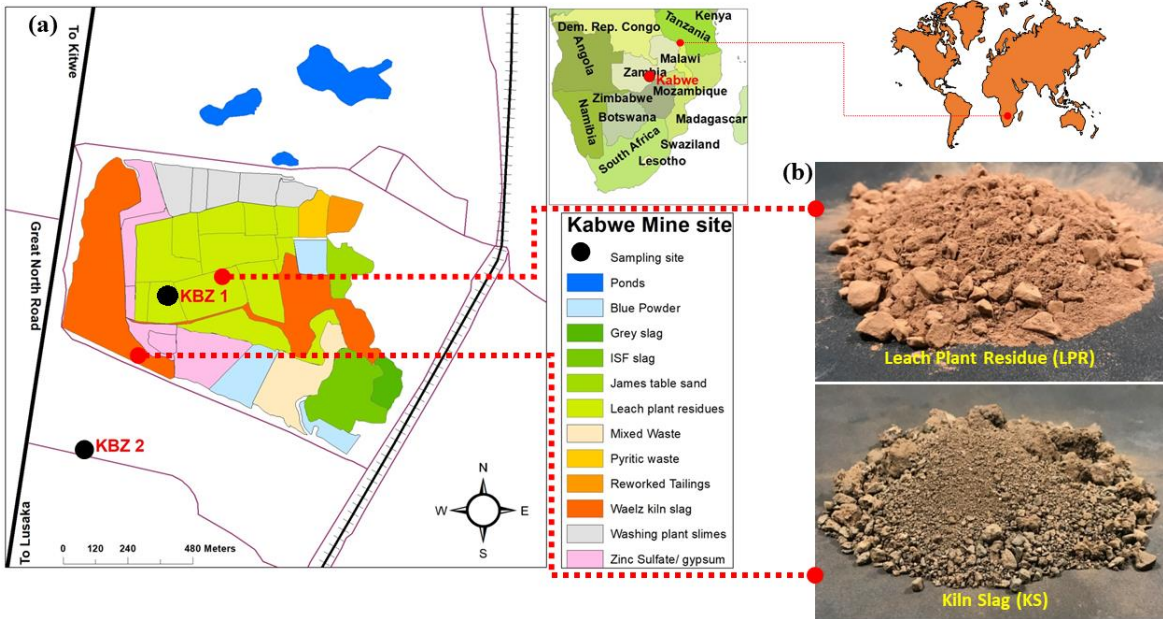
515   deviation of three independent replicates.

516   **Fig. 4.** Comparative view of control and biocemented sand obtained by the immersed and flow-

517   through methods facilitated by *O. profundus* KBZ 1-3 and *O. profundus* KBZ 2-5.

518   **Fig. 5.** SEM view and the corresponding XRD analysis comparing (a) control specimen, (b)

519   biocemented sand prepared by the flow-through method using *O. profundus* KBZ 1-3, and (c)

520   biocemented sand prepared by the flow-through method using *O. profundus* KBZ 2-5.

521   **Fig. 6.** Relationship between UCS and $CaCO_3$ content of sand biocemented by *O. profundus* KBZ

522   1-3 and *O. profundus* KBZ 2-5

523

(a)

To Kitwe

Great North Road

To Lusaka

KBZ 1

KBZ 2

0   120   240   480 Meters

N
W   E
S

Dem. Rep. Congo
Angola
Namibia
Zimbabwe
Botswana
South Africa
Lesotho
Swaziland
Zambia
Kabwe
Mozambique
Madagascar
Malawi
Tanzania
Kenya

Kabwe Mine site

- ● Sampling site
- Ponds
- Blue Powder
- Grey slag
- ISF slag
- James table sand
- Leach plant residues
- Mixed Waste
- Pyritic waste
- Reworked Tailings
- Waelz kiln slag
- Washing plant slimes
- Zinc Sulfate/ gypsum

(b)

Leach Plant Residue (LPR)

Kiln Slag (KS)

524
525   **Fig. 7.**
526

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527

528   Fig. 8.

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Fig. 9.

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Fig. 10.

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537 **Fig. 11.**

538

Fig.

12.
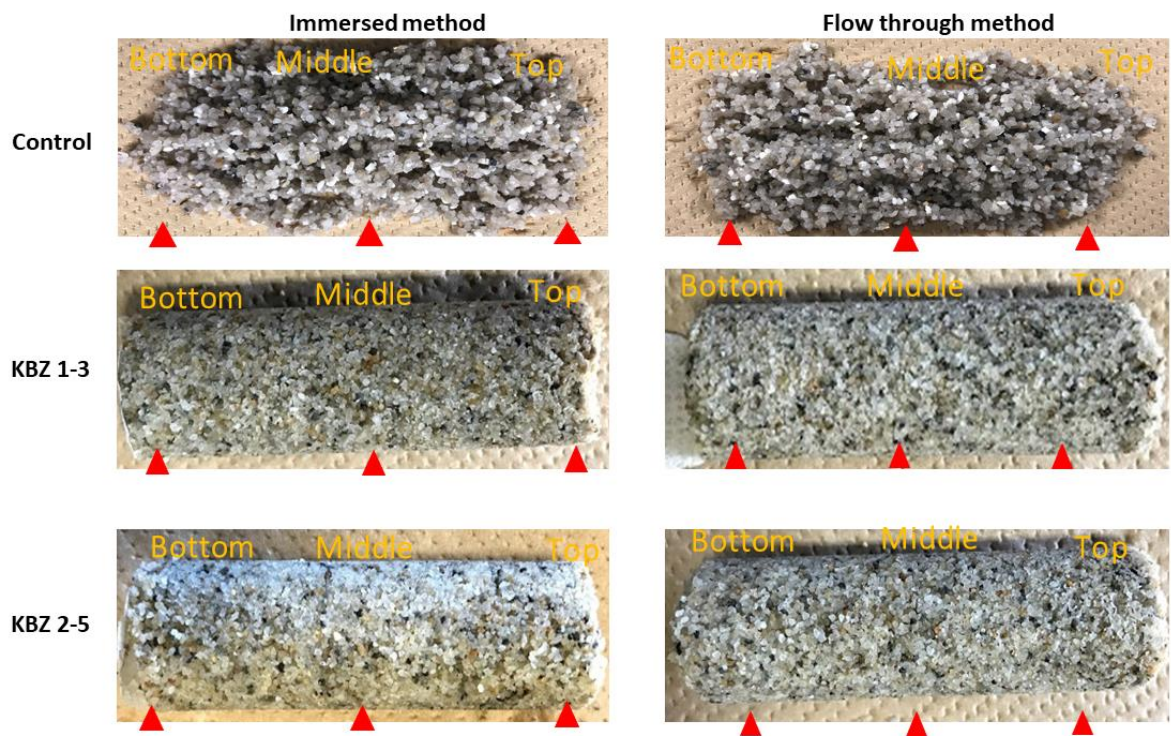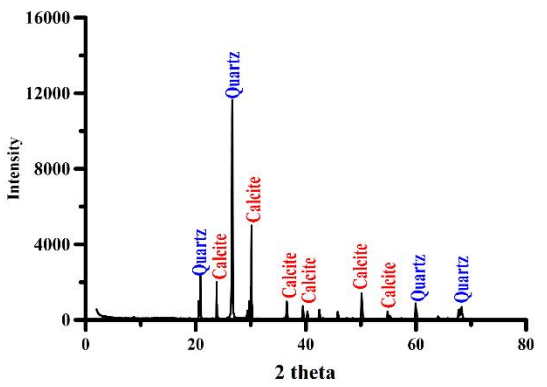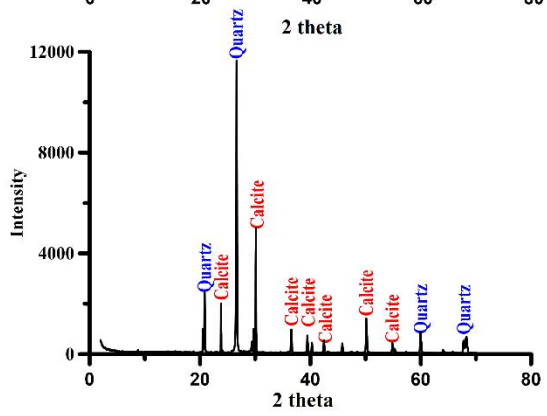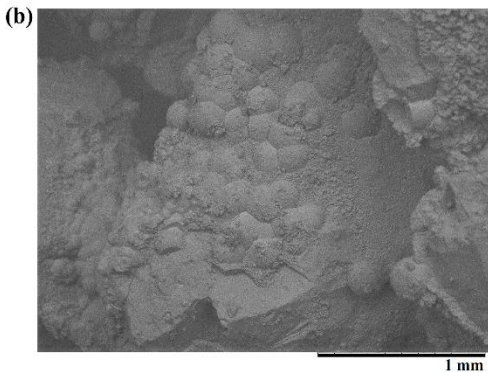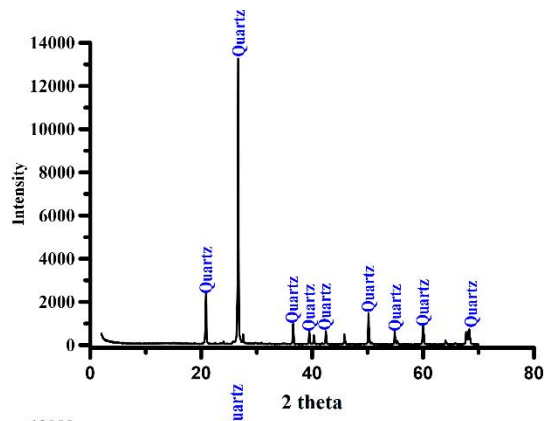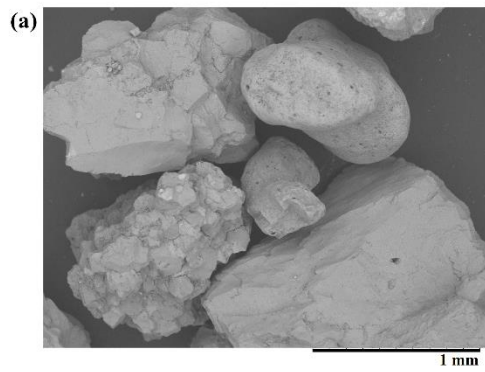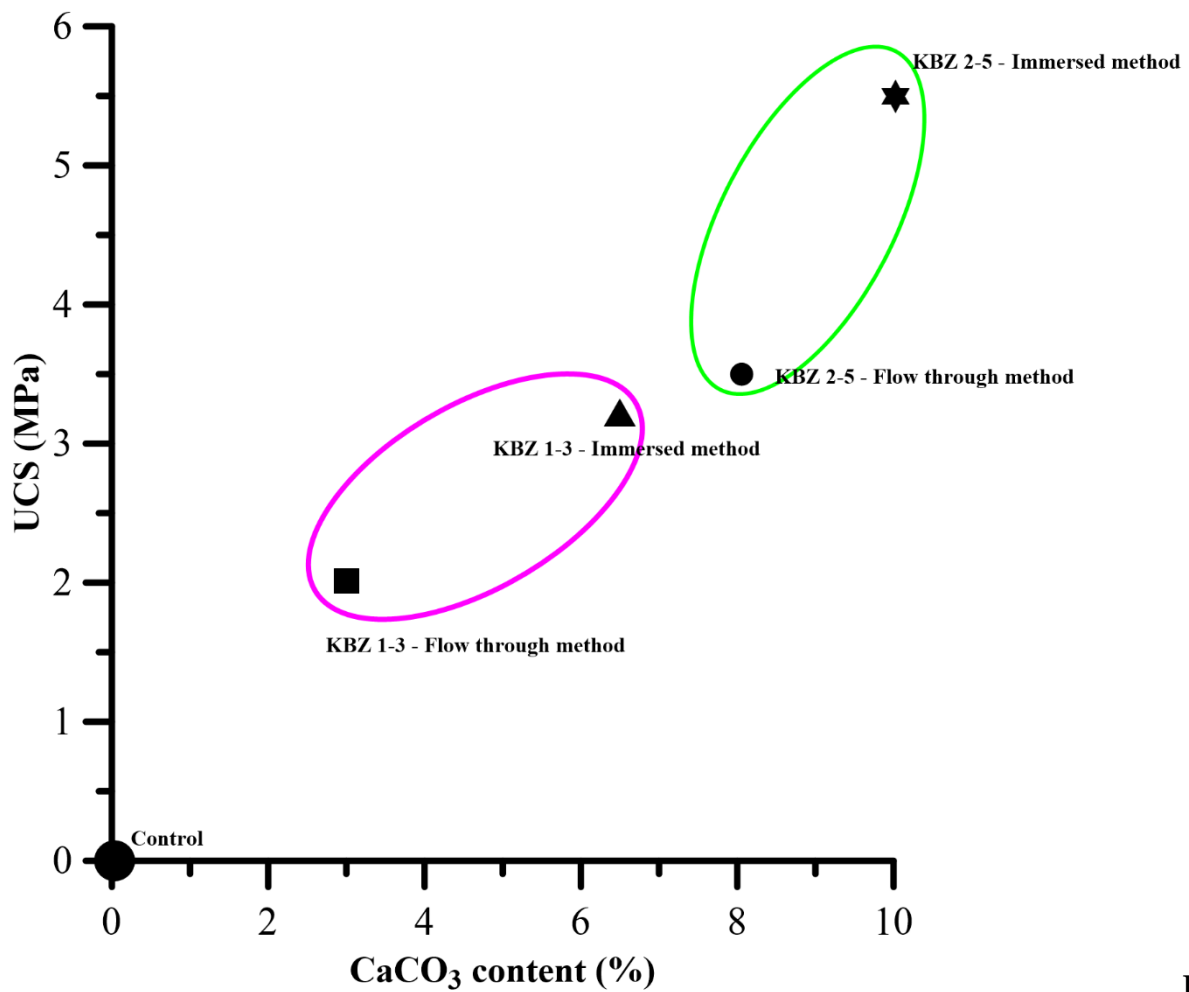
542 **Table 1.** Estimated UCS values of control and biocemented sand prepared by immersed and flow

543 through methods and mediated by *O. profundus* KBZ 1-3 and *O. profundus* KBZ 2-5

	Immersed method			Flow through method		
Specimen	Top	Middle	Bottom	Top	Middle	Bottom
Control	0	0	0	0	0	0
KBZ 1-3	3.2	1.8	1.4	2.0	1.4	1.0
KBZ 2-5	5.5	3.0	1.8	3.5	2.0	1.0

544

545