Research Article

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Effect of microbial-induced calcite precipitation (MICP) on the strength of soil contaminated with lead nitrate

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Abstract: Microbial induced calcite precipitation method MICP is a sustainable and eco-friendly technique for soil stabilization. To show the optimum effectiveness of the bioremediation within the silty sand matrix, a model of plastic boxes and PVC molds was made with an air pump placed in an isolated room at a temperature range of 25–27°C. The molds were perforated from sides and bottoms and opened from the top with a transparent film of filter paper (placed on the inner surface). The major feature of this treatment system is allowing the cementation solution to penetrate easily into soil samples. The results showed a positive effect of Bacillus subtilis in enhancing the strength properties of lead contaminated soil. Unconfined compressive strength increased from 65 kPa to 539, 527, and 525 kPa. Cohesion increased from 4.5 to 40, 41.9, and 42 kPa at concentrations of 15, 20, and 25% respectively.Angle of internal friction increased from 18.94° to 38.2°, 40°, and 40.74° respectively after 14 days. Thereafter, it become 40.92° and 41.5° at concentrations of 15 and 20%, respectively and decreased to 36.75° of 25% at 28 days. Microstructural characteristics represent the formation of calcium carbonate and lead compounds,which were the reasons for the improvement in the strength and the alteration in lead from a soluble to insoluble form, a hence less toxic element.

Keywords: silty sand, lead, Bacillus subtilis, MICP, direct shear, unconfined compressive strength

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1 Introduction

Over the past decades, the negative effects of conventional soil treatment technologies on the environment have encouraged soil researchers to put more emphasis on the implementation of eco-friendly soil modification approaches [1].

Heavy metals are regarded as important contaminants with a range of diverse effects on the geotechnical qualities of soils [2]. They impair the soil's engineering mechanical qualities, causing cracks in the building structure, settlement, and foundation deformation [3].

Although soils contaminants (particularly heavy metals) might restrict stabilization reactions and hence impair the strength development of stabilized soils, the content of the stabilizer may also show a significant role in the improvement of soil strength [4–6].

Currently, chemical, physical, and biological approaches are three accessible types of soil remediation technologies [7]. In terms of geotechnical applications, traditional soil improvement techniques require a lot of energy in the context of material production and on-site operation [8].

Bio-mediated soil treatment has just developed as a novel, long-term ground improvement technology. To create calcite in the soil matrix, this approach uses a biological process known as microbial calcite (MICP). The calcite produced is accountable for reinforcing and clogging soils [9]. The bacterial calcium carbonate precipitation is considered as an additive to modify the geotechnical properties of soils [10, 11]. MICP is a current and inventive technique of soil enhancement [12, 13]. MICP is a comparatively green, sustainable, and environmentally friendly strategy that can change and improve the ground state. Soil stabilization by the MICP technique satisfies the requirements of ecological construction as this treatment causes minimal disturbance in the soil environment [9, 14]. Bacteria and strengthening substances that penetrated into the soil to create calcite precipitation improves soil's engineering qualities [15].

The urease-driven MICP process is a promising selection for heavy metal immobilization. Throughout the precipitation of calcite, toxic metal ions can be incorporated into $CaCO_3$, substituting Ca^{2+} or co-precipitate in the $CaCO_3$ lattice structure [16].

With the use of the MICP technique in the bioremediation process, the role of calcium ion takes advantage of the Pb^{2+} incorporation on its surfaces through the substitution in the calcite lattice. Hence detoxifying the toxic lead ions, after which Pb^{2+} is changed from soluble to insoluble forms [16, 17].

The research aims are to demonstrate the influence of bacterial action on the geoenvironmental properties of lead contaminated silty sand. This experimental study was achieved by the biological deposition of calcium carbonate on silty sandy soil using Bacillus subtilis. The samples were prepared manually under two variables, including bacterial solution concentration and time of curing. The experiments included UCS and shear stress tests. Moreover, XRD, SEM analyses were applied to examine the microstructural modifications of the soil before and after treatment.

2 Materials and methodology

2.1 The soil

In this study, soil samples were brought from the Alkraiat region, north of Baghdad city, at a depth range from 0 to 1 m. The soil was oven dried, and sieved to ensure a uniform grade, then preserved at clean plastic bags prior to soil analyzing and contamination, were tested and analyzed according to (ASTM, D 422-63). The physical properties and soil classification are presented in Table 1, while the chemical test is illustrated in Table 2.

Table 1: Physical Properties of soil

Physical Properties	Value
Particle size distribution	
Sand (%)	55
Silt (%)	35
Clay (%)	10
Maximum Dry density (g/cm^3)	1.85
Optimum Moisture content (%)	12.2
Specific gravity	2.66
Soil classification USCS	Silty sand (SM)

Table 2: Chemical test of soil

Chemical test	Value (%)	Specification
Total Soluble Salt (TSS)	2.2	Earth manual E8
SO_3	0.81	BS 1377: Part 3: 1990
Organic Matter	1.286	
pH	8.39	
$CaCO_3$	22.95	
CL	0.096	
Gypsum ($CaSO_4 \cdot 2H_2O$)	1.64	

2.2 Microorganisms

An isolated bacterial entitled Bacillus subtilis was applied in this research, which is a Gram-positive, aerobic, rod-shaped, and spore-forming bacteria that can be found in water, soil, and plants [18]. It can induce $CaCO_3$ precipitation with appropriate media, as well as living in severe environments [19].

These bacteria were cultured in the Ministry of Science and Technology laboratories under aerobic batch conditions using a growth medium. The medium was prepared by dissolving the following chemicals in one liter of distilled water (Table 3). The medium solution was autoclaved for 12 minutes at 121°C.

Table 3: Growth media

Chemicals	Value
peptone	5 g
NaCL	5g
Yeast extract	4 g
Beef extract	1 g
Urea Solution	50 ml

Table 4: Cementation ingredients

Chemicals	Value
Nutrient broth	3 g
NH_4Cl (ammonium chloride)	10 g
$NaHCO_3$ (sodium acid carbonate)	2.12
$CaCl_2$ (calcium chloride)	27.75 g
$CO(NH_2)_2$ (urea)	30.03 g

2.3 Cementation reagent

Cementation reagents play a significant role in promoting calcite precipitation. The cementation solution ingredients per liter of deionized water are represented in Table 4.

2.4 The contaminate

In this study, type of contaminant used was lead nitrate (250 mg/kg), lead (400 mg/k), and lead (II) nitrate. The lead nitrate has high solubility in water and is readily dissolved in distilled water to give a colorless solution.

$$Pb(NO_3)_2(s) \rightarrow Pb^{+2}(aq) + 2NO_3(aq) \quad (1)$$

Then mixing by hand was performed until thoroughly homogenized, The Pb spiked soil was kept in clean plastic bags and allowed to physical and chemical behavior of lead through the soil for seven days.

2.5 Stirred tank reactor model

In this model, the MICP treatment consisted of 12 plastic boxes with dimensions (17 cm × 17 cm × 20 cm) and two models of molds for unconfined compressive strength and direct shear tests that were placed in each box (Figures 1 and 2). A split polyvinyl chloride (PVC) pipe (perforated from sides and bottom and opened from top, with a transparent film of filter paper) was placed on the inner surface to facilitate sample extraction and reduce potential losses of soil during preparation and treatment. The first model for measuring unconfined compressive strength with net inner dimensions of 10 cm length, 5 cm diameter, and a height to diameter ratio of 2:1. The second mold for measuring direct shear test with dimensions of 6 cm × 6 cm × 2 cm. To perform bio-mediated soil improvement, the prepared bacterial solution was mixed with the lead contaminated soil (15%, 20% and 25% by weight), and then the molds were distributed inside a plastic basin filled with the cementing solution. Two molds of each type were inserted in each box, generating a total number of 48 molds. The soil was extruded from the plastic mold, and a series of tests were performed to calculate the soil shear strength and unconfined pressure after 7, 14, 28 curing days.

The molds were placed on a perforated shelf inside the boxes. The boxes were filled with cementation medium, with a height of 2 cm less than the top. The boxes were connected to the aeration system: air pump, and plastic tubes that were extended into the boxes. The main procedure of this treatment system is as follows: soil samples were completely immersed in the cementing agent, the agent simply penetrated the soil samples. The temperature was controlled by placing the models in an isolated room (25–27°C), and measured periodically over time through the treatment period.

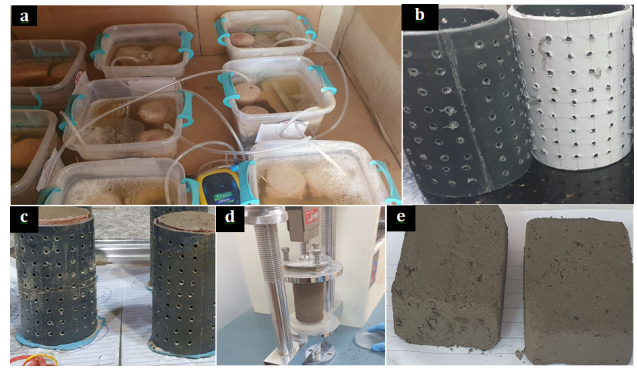

Figure 1: Photograph of the (a) Treatment system, (b, c, e) molds and samples, (d) UCS test

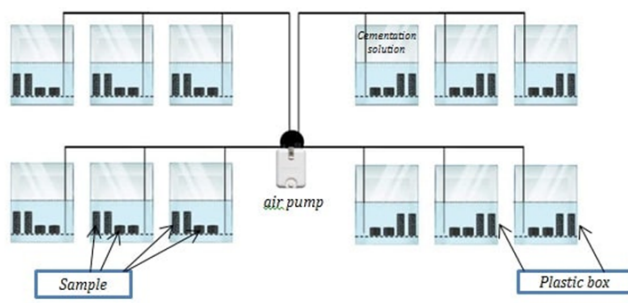

Figure 2: Schematic drawing for the treatment system

3 Results and discussion

3.1 The effect of MICP on the soil strength

3.1.1 Unconfined compressive strength (UCS)

In order to assess the improvement degree of soil stabilization, unconfined compressive strength (UCS) was used as a measure of strength characteristic of stabilized samples, which was an indication of whether the treated material had an acceptable strength to support any overburden pressure.

The test for silty sand soil before and after treatment was approved according to the system given in (ASTM D 2166-07). Figure 3 shows the UCS values of samples treated with different percentages of the bacterial solutions (0, 15, 20, 25%), which were obtained after 7, 14, and 28 curing days.

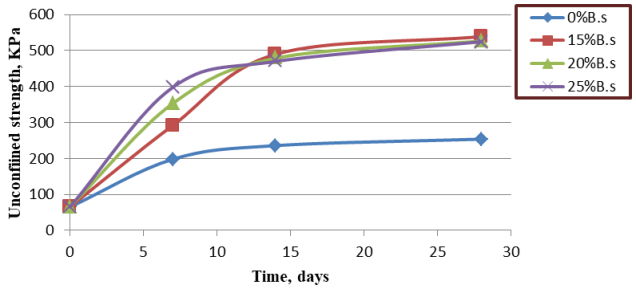

Figure 3: Unconfined compression strength versus time at different concentrations of bacterial solution (B.s)

It was observed that there was a clear increase in the compressive strength of polluted soil from 65 kPa to 539 kPa for sample with 15% bacterial solution at 28 days. Soil with 25% bacterial solution showed a sharper increase in the strength than that with 15 and 20% within the initial days, and growth becomes smooth until 28 days achieving a similar value, although the sample with a percentage of 15% showed 14 kPa higher than that with a percentage of 25%. This was due to the speed and quantity of carbonates formed, which fill the voids and act as linking bridges, or maybe due to a change in the concentration of cementing regent with time. Sharma and Ramkrishnan [20] found that UCS changed with cementing regent in spite of the bacterial solution, while Saing *et al.* [21] concluded that the value of compressive strength increases significantly along with the addition of bacteria and the increase in the curing time.

Also Hasriana *et al.* [22] found that the compressive strength value rose with the increase of bacteria but then declined. This phenomenon was due to several factors, i.e., Bacillus subtilis bacteria hydrolyze the urea which is catalyzed by the urease enzyme (produced by bacteria). With the dissolved Ca^{2+} in the surrounding environment, it produces solid calcite/calcium carbonate ($CaCO_3$) crystals that would bond closely and cause the soil hardness. The compressive strength value increases typically during the bacterial increase due to the increase in particle density caused by the Microbial-Induced Calcite Precipitation process. Meanwhile, the precipitated calcite is used to fill the cavity at soil mass and bond the weak soil grains. When the cavity decreases, precipitate calcite binds the solid soil. Therefore, when the concentration of bacteria increased, the value of unconfined compressive strength decreases.

The time factor had a clear effect on strength. Sharma and Ramkrishnan [20] found that strength increased positively with the treatment period. This is a reasonable outcome because the longer the treatment duration, the more calcium carbonate deposits are usually formed [23].

It was noticed that there was a slight growth in the compressive strength of the contaminated sample cured with the cementing solution (no bacterial solution), taking into account that literature indicated a decline in the compressive strength with a rise in the level of soil contamination [24], however Moghal *et al.* and Ojuri and Oluwatuyi [25, 26] showed an increase in the strength of polluted soil cured by cementing solution.

3.1.2 Direct shear test

The direct shear test was applied in this study to examine the behavior of shear strength parameters, cohesion (c) and angle of friction (Φ) for silty sandy soils before and after bacterial treatment at different durations (7, 14, and 28 days). The direct shear test was applied depending on the procedure given in ASTM D (3080-11).

Figure 4 represents the outcome of bacterial solution on cohesion (c) with time. It seems that the cohesion of natural soil increased with the increase of bacterial solution and curing time, from 4.5 kPa approaching 40, 40.9, and 42 kPa for soils containing 15, 20, 25% respectively of bacterial solution after 28 days. Also, the rate of increase is faster during the initial days for soil containing 25% bacterial solution [27]. Zamani and Montoya show that the MICP treatment method has a significant influence on shear strength properties of silty soil, as shear strength increases and excess pore pressures decrease. The shear stress values of the treated samples are greater than the untreated soil samples [10].

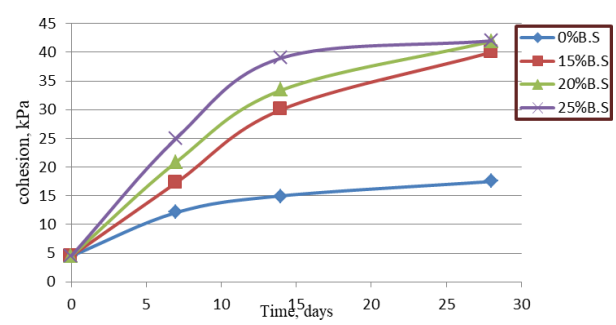

Figure 4: Influence of bacterial solution with time on the cohesion

Figure 5 shows the influence of bacterial treatment upon the internal friction angle of natural soil over time. It can be seen that there was an increase from 18.93° up to 38.2, 40.0, and 40.75° for contaminated soil with 15%, 20%, and 25% of bacterial solution respectively at 14 days of curing. Then, an increase up to 40.92 and 41.5° for bacteria solution of 15 and 20% with a little decrease to about 36.76° for 25% at 28 days. Montoya *et al.* [28] used MICP

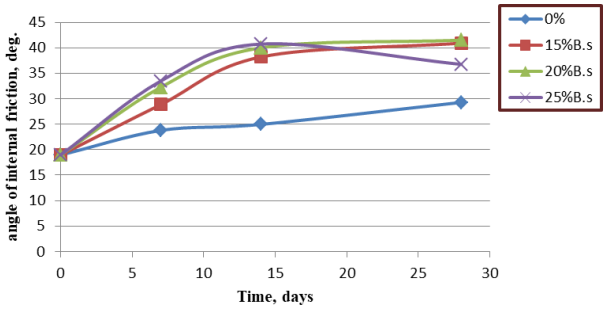

Figure 5: The effect of bacterial solution (B.s) with time on the internal friction

to reinforce sandy coastal deposits and found that the soil sample had a strength increase (as confirmed by the unconfined compression tests) and an increase in the friction angle. The slightly cemented sand had a resistance increase (as proved by the unconfined compression tests) and also an increase in the friction angle [27, 28]. In comparison, Pakbaz *et al.* [29] showed an increase in both (c) and (Φ) due to the MICP treatment.

There is a slight increase in cohesion (c) of the contaminated soil without bacteria cured in the cementing solution, so it increased up to 17.5 kPa, while the internal friction increased up to 29.28° at 28 days. This may be due to the chemical reactions between cementing solution and soil elements. The shear strength of lime-treated clay is a function of lime content. This is because the lime content affects the strength of expansion and hence expansion behavior of the cement joints [30]. However, Ali *et al.* [31] found that the cured lime-soil mixtures displayed a larger angle of friction' Φ' than the untreated soil, among three metal elements, lead was prominently dominating. The increase in the shear strength and friction angle of the stabilized soil is due to a production of the abovementioned cementitious materials in the soil-stabilizer matrix.

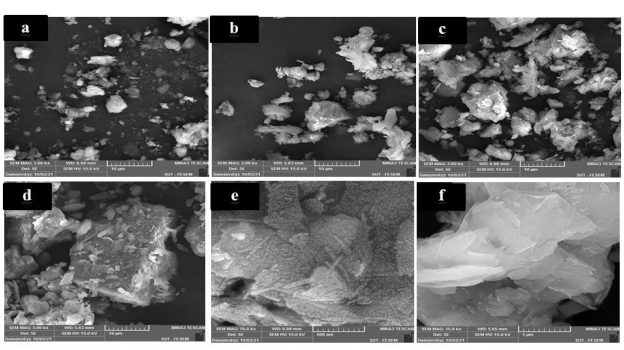

Figure 6: SEM confirm the presence of biomineralized crystal formations as a result of MICP (a) soil before treated, (b) lead polluted soil with Cementation reagent, (c, d, e, f) lead polluted soil treated with bacterial solution and Cementation reagent

3.2 Micro-structural characteristics

X-ray diffraction (XRD) and Scanning Electronic Microscope (SEM) tests were performed on the untreated and treated soil to supplement the experimental results and assist in the interpretation of the observed behaviors. Due to the formation of calcite crystals, SEM analysis revealed an increase in the matrix structure bonding that was confirmed by XRD analysis, with a significant decrease in the size of pores between particles. Figures 6 and 7 show SEM and

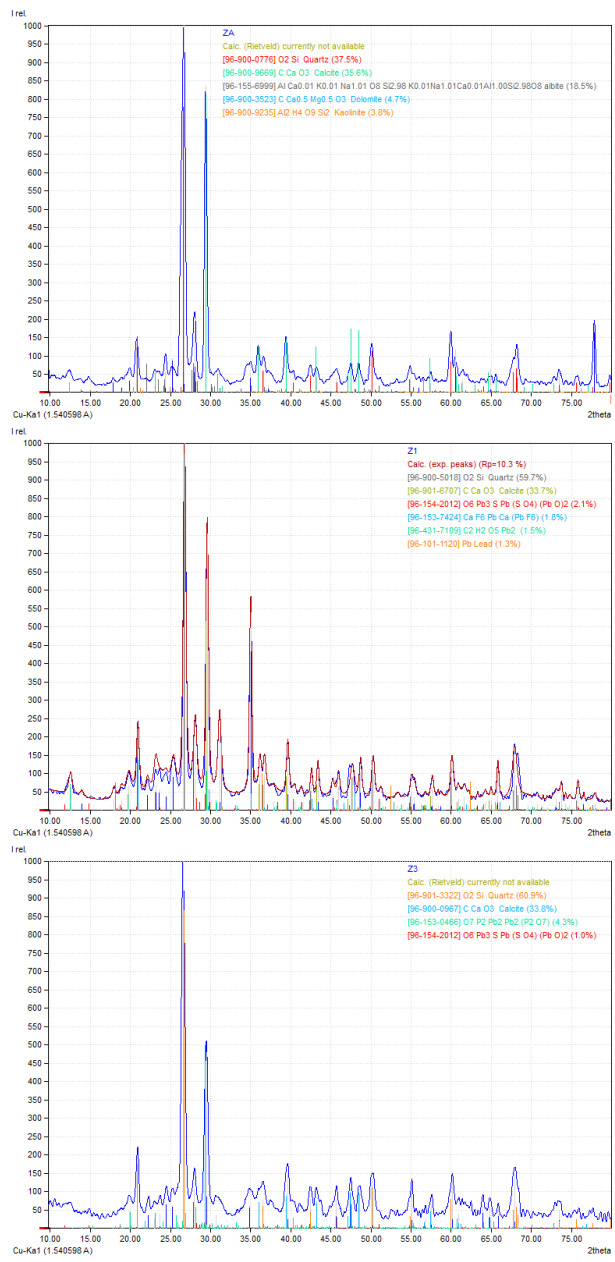

Figure 7: XRD of soil (a) soil before treatment (b) lead polluted soil with Cementation reagent (c) lead polluted soil with the bacterial solution and Cementation reagent

XRD, respectively. Toxic metal ions may be combined into $CaCO_3$ by substituting Ca^{2+} or may coprecipitate within $CaCO_3$ lattice structure [16].

4 Conclusions

One of the biological soil improvement techniques is the microbial-induced calcite precipitation (MICP). This research was performed to scrutinize changes in the engineering characteristics of lead polluted silty sand soil under two variables, including bacterial solution concentration and curing time. Throughout the study, the Bacillus subtilis bacteria had a positive effect on the soil geoenvironmental properties, which was observed by:

1. Unconfined compressive strength (UCS) increased for all concentrations of bacterial solution after 28 days of the treatment period even with the soil of 25% bacterial solution that represented a sharper strength increase than others within the initial days.
2. The cohesion increased with the increase in the bacterial solution and curing time.
3. The angle of internal friction increased with the increase in the bacteria solution and time up to 14 days, then keeping increased for bacterial solution of 15% and 20% with a slight decrease for 25% at 28 days, but higher than that before treatment.
4. Polluted soil, without bacterial solution, showed a slight increase in cohesion, unconfined strength, and angle of internal friction within curing time.
5. SEM and XRD analysis revealed an increase in the matrix structure bonding because of calcium carbonate production and lead compounds, which was responsible for the positive impact on soil strength and lead adaption from soluble to insoluble form resulting in a less poisonous element.

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