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ORIGINAL RESEARCH ARTICLE

Application of microbially induced calcite precipitation to mitigate soil frost heave

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ABSTRACT

BACKGROUND AND OBJECTIVES: Soil frost heaving causes significant destruction to road pavements, railways, pipelines, and other lifeline infrastructures. The conventional methods for dealing with the soil frost heave are primarily based on using the materials whose production and use are harmful to the environment. Due to the recent ecological concerns, developing novel alternative methods has received much attention. This study aims to investigate the possibility of using the microbially induced calcite precipitation method to control soil frost heave for less pollution introduction to the soil.

METHODS: In this study, the *Sporosarcina Pasteurii* bacterium was used for calcite precipitation. The influence of three factors in four levels, including bacteria concentration, cementing solution concentration, and curing time, was investigated based on a plan set by Taguchi design of experiment method. The results were obtained by analysis of means and analysis of variance statistical methods and compared with the conventional frost heave reduction methods.

FINDINGS: The results were presented in terms of heave ratio. Based on the testing results, the heave ratios (frost heave ratios of the treated to untreated samples) were obtained to be in the range of 0.21 to 0.42. The results showed that bacteria concentration was the most influential factor in the total frost heave of the treated soil. The influence of curing time was in second place, and the effect of cementing solution concentration was relatively less. The minimum frost heave was achieved in 10^8 colony-forming units per milliliter bacteria concentration, 0.6 mole per litre cementing solution concentration, and 21 days of curing.

CONCLUSION: The findings indicated that the used method could be efficiently used to reach the desired objective. The heave ratios obtained by this method were promising to a great extent compared to the conventional methods. The reduction of frost heave due to the application of this method was attributed to the precipitated calcite within the soil voids and was justified by the scanning electron microscopy images of the treated soil samples. This study proved that the proposed method might be utilized as a potential ecological-friendly approach in the future researches.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
50	9	6

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INTRODUCTION

Soil frost heaving occurs when several factors, including water and frigid weather, are simultaneously available in the presence of susceptible soils in civil engineering projects. The most influential factors in frost heave are soil particle size distribution (Qi *et al.,* 2022), dry density (Zhao et al., 2019), plasticity index (Isik *et al.,* 2013), fines percent (Wu *et al.,* 2018), groundwater level (Lin et al., 2018), moisture content (Mao and Zhang, 2018), and water solutes (Shah and Mir, 2022). When the water is under significant forces from soil particles, it will freeze at lower temperatures than normal freezing water temperatures. The excess water would be absorbed from other zones due to the capillary effect and via heat flow simultaneously, and the ice lens grows, leading to soil swelling (Bai *et al.,* 2020). In this condition, the soil frost heave happens and develops with the connection of the freezing zone to the external water sources (Fig. 7a). This detrimental phenomenon causes a significant damage occurring seasonally and periodically to roads, pavements, railways, buildings, and other lifeline infrastructures such as pipelines. Dealing with this phenomenon in susceptible areas is vital, and the solution must be both economically justifiable and eco-friendly. Thus, using novel methods to deal with this problem can be important for geotechnical engineers. Some studies and many real-life projects have been done to control and mitigate the soil frost heave and its effects. Frost heave mitigation can be met through bounding soil particles, filling the voids among particles, disbanding soil particles, and changing the fluid characteristics among particles (Lambe, 1956) (Fig. 7b). In one of the most comprehensive and early studies, Lambe, (1956) introduced 40 different additives to the sample soils and presented the treatment effect in terms of heave ratio. He also evaluated resin-type additives, portland cement, cations, dispersants, and water proofers on the frost heave of three soil samples. Kettle and McCabe, (1985) investigated the effect of mechanical stabilization using slag, basalt, and limestone as stabilizing agents, which were divided into two particle groups for their gradation to control frost heave. They reported that the slag aggregate led to the highest heave reduction and the increased coarse-aggregate content was directly related to the performance of the used method. Zieba *et al.* (2019) applied micro-silica and nano-silica to sandy clayey silt (saclSi) which is frost-susceptible soil. They showed that adding 5% nano-silica could lead to an almost complete reduction in frost heave. They also reported that mixing the same amount of micro-silica reduced the frost heave by 21%. Liu *et al.* (2022) indicated that the frost heave amount can be mitigated by electro-osmosis dewatering. A growing trend is forming to limit the application of conventional additives, such as chemical grouts, lime, and cement, for ground improvement purposes, including frost heaving control. This limitation stems from the fact that materials production and use have been proven to be harmful to the environment. According to Karol, (2003), almost all chemical grouts are toxic and hazardous to nature. Thus, many researchers have pursued the growing prospect of developing new safe ground improvement methods. Among the new ground improvement techniques in some fields of civil engineering, such as self-healing concretes (Muhammad *et al.,* 2016) in structural engineering and soil improvement in geotechnical engineering (Shahrokhi-Shahraki *et al.,* 2015), microbially induced calcite precipitation (MICP) seems to be a promising method, particularly from an environmental point of view. This method, however, faces some challenges including ammonia by product (Lee *et al.,* 2019). In this context, some soil native microbes are triggered to procure rapidly and create calcite cement within the soil matrix, which enhances the soil's hydro-mechanical properties. Several bacteria, such as *Proteus mirabilis* and *Proteus vulgaris mixture* (Talaiekhozani *et al.,* 2014), *Bacillus sphaericus* (Hataf and Baharifard, 2020), *Sporosarcina pasteurii* (Han *et al.,* 2016), and *Bacillus subtilis* (Wath and Pusadkar, 2021) have been used in the MICP procedures. Among these bacteria, *Sporosarcina pasteurii* has been suggested as the most efficient bacteria for the MICP process in soil (Rahman *et al.*, 2020). *Sporosarcina pasteurii* is a non-pathogenic bacterium capable of producing high amounts of enzyme urease (Henze and Randall, 2018). These rod-shaped, gram-positive bacteria precipitate calcite if suitable conditions are provided. In the most common procedure, calcite needs to precipitate within soil pores, cementite the soil, and bound its particles together. This calcite is produced by hydrolysis of urea via urease enzyme, which occurs in a calcium-rich environment (Castanier *et al.*, 1999). The reactions in which this process occurs using Eqs.

1, 2, 3 and 4 (Deng *et al.*, 2021).

$$(NH_2)_2CO + H_2O \rightarrow 2NH_3 + CO_2 \quad (1)$$

$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^- \quad (2)$$

$$CO_2 + OH^- \rightarrow HCO_3^- \quad (3)$$

$$Ca^{2+} + HCO_3^- + OH^- \rightarrow H_2O + CaCO_3 \quad (4)$$

Many studies have been performed on improving soil properties using the MICP approach. Modification of soil compressive strength (Xiao *et al.*, 2021), increasing the soil shear strength (Zamani and Montoya, 2017), reducing soil permeability (Roth and Caslake, 2019), creating waste containment barriers (Etim *et al.*, 2022), mitigating liquefaction (Zamani *et al.*, 2021), and increasing the shear capacity of the soil-steel interface (Bak *et al.*, 2021) are among these studies. In general, application of the biological soil improvement technique is a very promising method. It can also be considered as a suitable alternative to conventional methods and reduce the harmful environmental effects. Some studies have shown the significant ability of calcite precipitation in various aspects of soil improvement as it increases the bounding of soil particles and reduces the voids among them. Various applications of the MICP method have attracted the attention of many researchers till now, and different aspects of this approach are becoming more apparent. The aim of this study was to evaluate the possibility of using this approach to improve, control, and mitigate the soil frost heave for the first time. It was also attempted to assess the Influence of three essential factors, including bacteria concentration, cementing solution concentration (CSC), and curing time, which could affect this phenomenon. For this purpose, a research testing program was developed by Taguchi design of experiment method. A frost heave testing apparatus was designed and built to assess the frost heave in the soil samples. The *Sporosarcina pasteurii* bacterium was selected to proceed with the MICP procedure as approved in the previous studies. The results were analyzed for the influence of each factor and their optimum level tested by ANOVA and ANOM statistical methods. The findings approved the efficiency of the adopted method as the frost heave in the soil treated with optimal bacterial culture was reduced to nearly one-fifth of the untreated soil. The novelty of this study lies behind successful application of the MICP approach to control soil frost heave for the first time. This revealed the MICP's capacity for development and industrialization in the future. This study was performed in Isfahan, Iran, during 2020-2022.

MATERIALS AND METHODS

Microorganism and cultivation

Sporosarcina pasteurii American Type Culture Collection (ATCC) 6453 was used to accomplish the MICP procedures on a silty soil identified as soil with frost heave potential. All the culture mediums used for bacterial cultivation were the same. To prepare the culture medium for bacteria cultivation, 13 g of nutrient broth powder was mixed in 1 L distilled water and autoclaved for 20 minutes (min) at 121 °C. After cooling the medium, 20 g/L urea sterilized by a Polytetrafluoroethylene (PTFE) filter was added to it. The bacteria strains were provided in a lyophilized form. The lyophilized powder was solved in 40 milliliter (mL) of the above medium, cultivated overnight in a shaking incubator at 30 °C, and centrifuged at 120 rpm to activate the bacteria. Then the solution was subcultured two more times with the same culture medium. To safely store the bacteria, 900 mL of cultivated bacteria and 600 mL of glycerol solution were mixed in some vials, and the obtained bacterial stocks were kept in a -80 °C freezer.

Bacterial transfer solution

As suggested by Stocks-Fischer *et al.*, (1999), a solution was made containing 3 g/L nutrient broth, 10 g/L of ammonium chloride (NH_4Cl), and 2.12 g/L sodium bicarbonate ($NaHCO_3$) autoclaved for 20 min in 121 °C. Using syringe PTFE filters, 20 g/L sterilized urea was added to the solution while its pH was adjusted at 6. The prepared solution was called bacterial transfer solution. For the primary cultivation of bacteria, a sample subcultured three times from the bacterial glycerol stocks was poured into a culture medium comprising sterilized nutrient broth and 20 g/L sterilized urea using syringe PTFE filters and was cultivated overnight. The outcome was fresh bacteria mixed with metabolic supernatants. The obtained solution was centrifuged for 25 min at 4000 rpm to harvest the bacteria. After removing the supernatant, the sedimented bacteria was mixed with sterilized physiological saline made of distilled water and NaCl.

The solution was re-centrifuged with the same profile to remove all impurities. This step was repeated two times, and the pure bacteria was then mixed with an appropriate amount of the injection solution to reach the desired bacteria concentration. The bacteria concentration was adjusted by a spectrophotometer, which measured the absorbed light of solution in a specific wavelength, in order to calculate the concentration of the bacteria. For the wavelength of 600 nm, the bacteria concentration was calculated using Eq. 5 (Okwadha and Li, 2010).

$$\mathrm{CFU/mL} = 8.59 \times 10^{7} \times \mathrm{OD}_{600}^{1.3627} \quad (5)$$

To determine the viable bacteria count and verify the spectrophotometer data, some samples were taken from the final solutions and cultivated on a petri dish filled with a culture medium consisting of sterilized nutrient agar and 20 g/L sterilized urea using syringe PTFE filters. The bacteria concentration was later estimated using the Miles method (Miles *et al.*, 1938).

Cementing solution

To trigger and maintain the MICP process at an adequate rate, a calcium-rich environment and a sufficient amount of urea are necessary for continuous urea hydrolysis. These substances are usually provided continuously to prohibit the termination of the MICP processes and end their reactions. The referred substances are provided in different forms and concentrations. In this study, the cementing solution consisted of anhydrous calcium chloride (110.98 g/M) sterilized by 20 min autoclaving at 121 °C and urea (60.06 g/M) sterilized by PTFE filters. According to previous studies (Whiffin *et al.*, 2007), the calcium chloride and urea concentrations were considered equimolar for all the cementing solutions.

Table 1: Specifications of the testing soil

Composition	Amount
G_S	2.69
D_{50}	0.1 (mm)
$\gamma_{d\,max}$	17.4 (kN/m^3)*
$\gamma_{d\,min}$	14.9 (kN/m^3)
C_u	2.2
C_c	1.16

*Kilonewton per cubic meter

Testing soil

Frost heave susceptible soils contain fine particles, especially silt, and the probability of frost heave occurrence increases with the increase of silt percentage. The soil particle size analysis was performed according to the ASTM D2487-17 standard and categorized using the Unified Soil Classification System. The sieve analysis testing results related to the selected soil are presented as a grading curve in Fig. 1. The soil sample was classified as SM in the unified soil classification system. The sample soil contained 24% cohesionless fines. Such soil was prone to frost heave occurrence. Some of the soil specifications are summarized in Table 1.

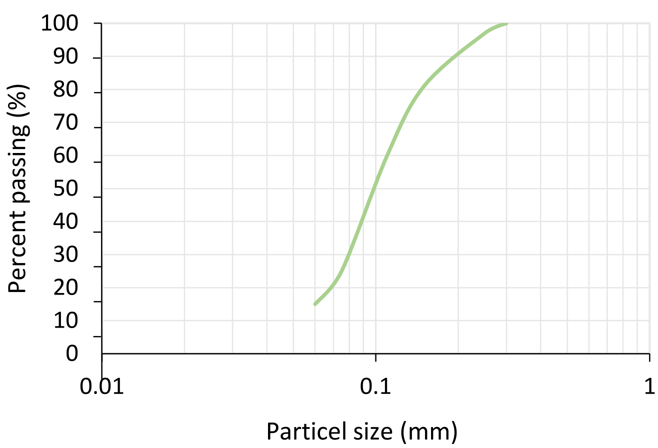

Fig. 1: Particle size distribution of the testing soil

Frost heave testing apparatus

A set-up of frost heave testing apparatus was designed and built considering the characteristics required to measure the amount of soil frost heave and also the data needed to proceed. The apparatus consisted of some main parts, including a specimen cylinder, top and bottom caps, top cap cooling and bottom cap heating parts, a saturation tank, and a processor that controlled the specimen cylinder's temperature gradient and recorded the data from sensors. The sections of the testing apparatus are shown in Fig. 2. The specimens were prepared in an acrylic cylinder with 120 mm diameter and 350 mm height. The bottom and top caps were made of copper plates for rapid and better heat exchange. Thermoelectric coolers were mounted at the top cap of the apparatus, Thus, controlling the temperature of the top cap could be made more precisely as compared to traditional methods. These thermoelectric coolers could quickly reduce the temperature of the top cap as low as -25 °C. The movement of the upper cap was restricted to move only in the vertical direction by two shafts connected to the main frame using a restricting bearing system. The bottom of the cylinder was closed with the bottom cap, sealed, and fixed in its place. A heating element controlled by an appropriate processor was used for heating the bottom cap in order to achieve the desired temperature. The processor got its data from three sealed digital temperature sensors placed at the specimen's top, middle, and bottom (with 10 cm spacing intervals). These sensors controlled the temperature of the top and bottom caps by two separate control systems to attain the temperature gradient set by the user. The processor recorded the data of the displacement sensor, time, and the step number in each step on a memory card with two-second steps. It also sent this data to the computer through its USB port connector. Strong insulation was employed to cover the acrylic cylinder in order to prevent the heat exchange of the perimeter of the cylinder with open air. The saturation tank was connected to the lower cap, and the water intake of the frost heave was supplied from the water of this tank.

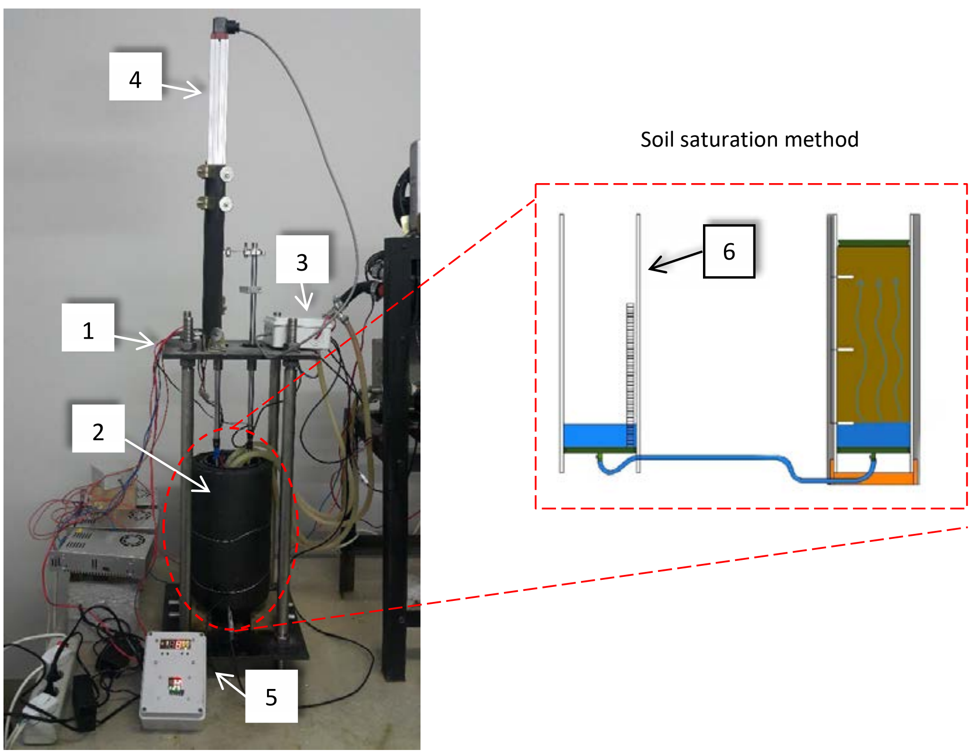

Fig. 2: Frost heave testing apparatus: main frame (1), insulated specimen cylinder (2), data processor and recorder (3), displacement sensor (4), temperature control panel (5), and water tank (6)

Experimental procedure

Specimen preparation

The first step for sample preparation was to clear the soil from any likely available bacteria that could affect the test conditions. For this purpose, the required amount of the testing soil (3000 g) was poured into an appropriate container and put in an oven at 120 °C for 24 hours. After cooling to the laboratory temperature, 600 mL of the transfer solution containing bacteria was mixed well with the soil sample. To prevent fine particles from washing out of the soil sample, a suitable filter was placed on the bottom cap of the cylinder. Moreover, the inner side of the acrylic cylinder was lubricated with silicone grease to prevent the adhesion of ice lenses to the wall and guarantee the free vertical movement of the soil sample. The prepared soil was then poured into the cylinder and compacted into five equal lifts with a thickness of 4 cm for each layer to obtain the same density of 1.63 g/cm^3 for all the specimens. After placing a filter at the top of the sample, the first cycle of injecting cementation solution into the sample was carried out from the top of the cylinder using 600 mL of the solution at a rate of 0.8 mL/min. The second injection cycle was started 12 hours after the end of the first cycle, using another 600 mL of cementation solution at the same rate. Based on previous studies, the specimens were cured under a constant temperature of 28 °C for all considered curing times (Khaleghi and Rowshanzamir, 2019). To explore the effects of the curing time on the performance of treated samples, the specimens were cured for various periods of 0, 7, 14, and 21 days before being subjected to frost heave testing. After curing, the testing specimen was washed with sterilized water injection to stop all the metabolic processes and then dried thoroughly.

Testing method

The prepared specimen was then placed into the frost heave test apparatus, and the saturation tank was connected to the valve of the bottom cap of the testing cylinder. The water level at the lower part of the testing sample played an essential role in the soil frost heaving procedure, as reported in previous studies (Hermansson and Guthrie, 2005). The water level within the testing sample was maintained at a height of 5 cm relative to the bottom cap for all the considered specimens. To simulate ambient natural conditions, no extra saturation was imposed to the specimens. The moisture within the specimen was achieved through capillary water rise from the bottom connection to the water tank with an adjusted stable water level. The hydrostatic water pressure constantly maintained a water level with 5 cm height within the sample through the connected saturation tank until there was no change in the water level in the tank. To conduct the frost heave test, the temperature of the top cap was adjusted to -25 °C, and the bottom cap was kept at 4 °C. This temperature condition provided a 1.05 °C/cm temperature gradient for the testing specimen. The test run for 35 hours to allow the testing specimen to reach a steady state.

Design of experiment

Various factors affect the frost heave phenomenon and the MICP treatment procedure. Nevertheless, some factors have more influence on the final result and must be considered more precisely. Considering the previous studies (Khaleghi and Rowshanzamir, 2019), bacteria concentration, CSC, and curing time were selected as most influential factors to be used in the present study. All the three factors were considered at four different levels in the designed tests (Table 2). However, other factors and parameters of the experiments were kept constant for all tests.

Taguchi's design of experiment (TDOE) (Taguchi *et al.*, 2005) was chosen to be used for planning the tests, due to its efficiency in reducing the number of required tests and its precise and reliable results. Each test ran within the given factor and level until achieving three logical repetitions. If an ambiguous result was obtained, the same experiment would be repeated until reaching a trustworthy outcome. The

Table 2: Different levels of the studied factors

Variable Factor	Level 1	Level 2	Level 3	Level 4
Curing period (day)	0	7	14	21
CSC (M)	0.3	0.6	0.9	1.2
Bacteria concentration (CFU/mL)	10^5	10^6	10^7	10^8

Table 3: MICP treatment test program designed by Taguchi method

	Input factors		
Test No	Curing times (day)	CSC (M)	Bacteria concentration (CFU/mL)
T1	0	0.3	10^5
T2	0	0.6	10^6
T3	0	0.9	10^7
T4	0	1.2	10^8
T5	7	0.3	10^6
T6	7	0.6	10^5
T7	7	0.9	10^8
T8	7	1.2	10^7
T9	14	0.3	10^7
T10	14	0.6	10^8
T11	14	0.9	10^5
T12	14	1.2	10^6
T13	21	0.3	10^8
T14	21	0.6	10^7
T15	21	0.9	10^6
T16	21	1.2	10^5

experiment was designed using Minitab statistical software (20.4 version) in Taguchi's L16 orthogonal array framework. The developed testing program is provided in Table 3.

Data analysis

To further analyze the tests results, first the signal-to-noise ratio (SNR) were calculated via the less is better assumption. The SNR equation can be presented as Eq. 6 (Mortazavi Bak *et al.*, 2021).

$$SNR = -10 \times \log\left(\sum_{i=1}^{n} (FH_i)^2 / n\right) \tag{6}$$

Where, n is equal to three as each test was repeated for three times. After calculating the SNRs, the obtained data were put under the analysis of means (ANOM) to identify the optimum level of each factor for achieving the minimum frost heave amount. For this purpose, the mean SNR ratio was calculated according to Eq. 7 (Mortazavi Bak *et al.*, 2021).

$$\text{Mean SNR} = \frac{1}{z}\sum_{t=1}^{z}(\text{SNR})_t \tag{7}$$

Where, z is the selected to be four earlier. To determine the mean SNR for each factor level, the obtained SNRs for that level were inserted in Eq. 7.

The analysis of variance (ANOVA) was also performed to determine contribution percent (CP) of each factor and p-value (Frossard and Renaud, 2021). The CP of each factor is calculated based on Eq. 8-12 (Bak *et al.*, 2021).

$$CP = \frac{SS - (DOF \times V_{er})}{SS_T} \times 100 \tag{8}$$

$$SS = \frac{mn}{Z}\sum_{z=1}^{Z}(\bar{X}_z^{\,f} - \bar{X}_T)^2 \tag{9}$$

$$SS_T = \sum_{j=1}^{m}\left(\sum_{i=1}^{n} X_i^2\right)_j - mn(\bar{X}_T)^2 \tag{10}$$

$$\bar{X}_T = \sum_{j=1}^{m}\left(\sum_{i=1}^{n} X_i\right) / (mn) \tag{11}$$

$$V_{er} = \frac{SS_T - \sum_{f=1}^{F} SS}{m(n-1)} \tag{12}$$

The test results in a set of data revealed that the sample heave magnitude with time was the most important case. Typical diagrams, including the one shown in Fig. 3, were plotted for each tested sample based on the acquired data. These diagrams

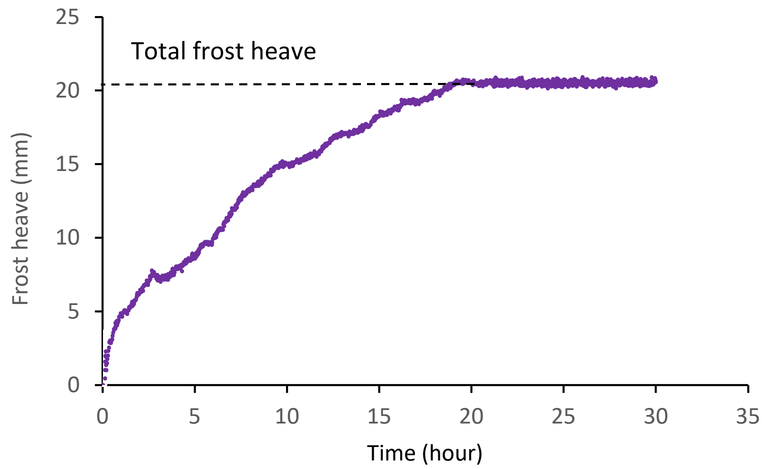

Fig. 3: Diagram of time and frost heave for the untreated soil sample

illustrated the frost heave magnitudes versus time and provided the required knowledge to investigate the process of frost heaving for each sample. During the testing period, each specimen reached a steady state in which the frost heave tended to a constant limiting value with no more change with time. This maximum limiting value of heaving was considered as the total frost heave value for the testing sample based on which the frost heave performance of the sample was processed to analyze the experimental results.

RESULTS AND DISCUSSION

The first test was conducted on an untreated soil specimen without performing any stabilization measure. This test was performed for two more times to assess the precision of the measured frost heave. The recorded heaves from these repetitions were 20.48, 20.51, and 20.60 mm, indicating high precision in the measured data. Moreover, the mean total frost heave for the untreated soil was obtained as 20.5 mm. Subsequently, the frost heave tests were conducted on the microbially treated soil samples (Table 3). The testing results are summarized in Table 4, which introduces the results from the defined tests derived from three repetitions (columns 5-7). Column (8) of Table 4 gives the mean value of the soil sample frost heave which is obtained from three repetitions for each testing case. These mean values were used to determine Taguchi predictions for all the designed testing cases presented in column (9) of Table 4. Column (10) gives the values for Taguchi predictions in column (9), divided into the frost heave value of the untreated samples. Finally, column (11) of Table 4 presents the SNR values calculated from Eq. 6.

Table 5 presents the data derived from Table 4 and Eq. 7. Table 5 includes the amounts of mean SNRs for all the factors considered in the experiment program. The SOP was obtained by subtraction of the maximum and minimum levels of each parameter.

The smaller mean SNR of a level for a test factor of the experiment implied that the factor level had a higher influence on the obtained result. Moreover, the significance of the parameters for each factor demonstrated the impact of the parameters on the measured frost heave. For more clarification, the data in Table 5 were plotted in Fig. 4. Obviously, the optimum conditions occurred within the 4th level (21 days) of curing time, 2nd level (0.6 M) of CSC, and 4th level (10^8 CFU/mL) of bacteria concentration leading to the lowest possible frost heave.

The ANOVA statistical analysis was performed using Eq. 8-13 to determine the contribution percent of each factor (Table 6).

The ANOVA results in Table 6 also demonstrated that bacteria concentration had the most significant effect (41.69%) on frost heave control. The curing times was in the second rank, with nearly the same influence (40.75%) on the test outcome as bacteria concentration. The CSC had the least effect (12.90%) on the frost heave reduction. The obtained

Table 4: **Frost heave test results and Taguchi predictions of MICP treated soil samples**

Test No.	Parameter level			Frost heave (mm)						
	Curing time	CSC	Bacteria concentration	First repetition	Second repetition	Third repetition	Mean $(\overline{FH})$	Taguchi prediction	Heave ratio	SNR
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
T1	1	1	1	8.36	8.4	8.47	8.41	8.61	0.42	18.50
T2	1	2	2	7.1	7.04	7.07	7.07	7.12	0.35	16.99
T3	1	3	3	6.79	6.82	6.70	6.77	6.79	0.33	16.61
T4	1	4	4	7.03	6.99	6.98	7.00	6.74	0.33	16.90
T5	2	1	2	7.40	7.39	7.35	7.38	7.43	0.36	17.36
T6	2	2	1	7.55	7.56	7.6	7.57	7.28	0.36	17.58
T7	2	3	4	5.51	5.56	5.55	5.54	5.82	0.28	14.87
T8	2	4	3	6.69	6.75	6.75	6.73	6.70	0.33	16.56
T9	3	1	3	6.13	6.08	6.09	6.10	6.06	0.30	15.71
T10	3	2	4	4.57	4.59	4.64	4.60	4.78	0.23	13.26
T11	3	3	1	6.85	6.79	6.82	6.82	6.65	0.32	16.68
T12	3	4	2	6.38	6.35	6.38	6.37	6.40	0.31	16.08
T13	4	1	4	5.59	5.60	5.61	5.60	5.40	0.26	14.96
T14	4	2	3	5.02	4.97	4.98	4.99	5.05	0.25	13.96
T15	4	3	2	5.91	5.87	5.92	5.90	5.78	0.28	15.42
T16	4	4	1	6.59	6.63	6.61	6.61	6.87	0.34	16.40

Table 5: Mean SNRs of the parameters at different levels

	Mean SNR				
Parameter	Level 1	Level 2	Level 3	Level 4	SOP
Curing time	17.27	16.64	15.52	15.33	1.94
CSC	16.69	15.57	15.91	16.58	1.12
Bacteria concentration	17.33	16.45	15.77	15.20	2.13

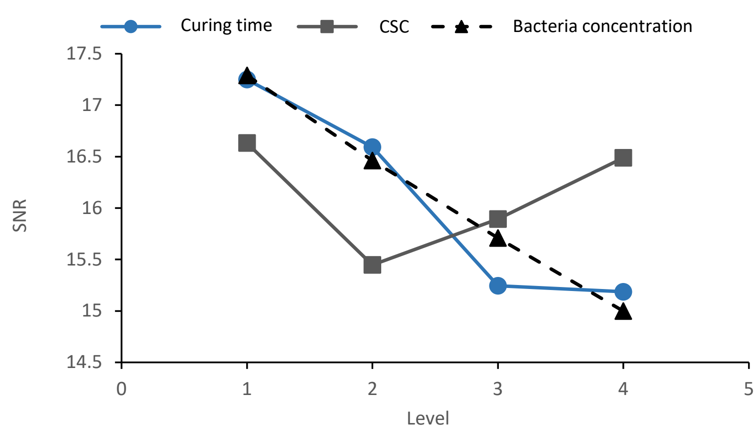

Fig. 4: Mean SNRs at different levels of each factor

p-values were less than 0.05, which rejected the null hypothesis (Andrade, 2019). The results of mean frost heave (column 8) and Taguchi predictions (column)) for T1 to T16 from Table 4 are plotted in Fig. 5. The R^2, which is calculated by Eq .13, is equal to 0.97, showing that the outcomes were firmly dependent on each other (Chicco *et al.*, 2021). The precision of the TDOE method was previously proved in previous studies (Lafifi *et al.*, 2019). This was in agreement with the results presented in the current study. Thus,

Table 6: ANOVA results

Factor	DOF	SS	MS	CP (%)	p-value
Curing time	3	11.39	3.80	40.75	0.002
CSC	3	3.60	1.20	12.90	0.037
Bacteria concentration	3	11.65	3.88	41.69	0.002
Residual Error	6	1.30	0.23	4.66**	
Total	15	27.94*		100.00	

*SS_T **V_{er}

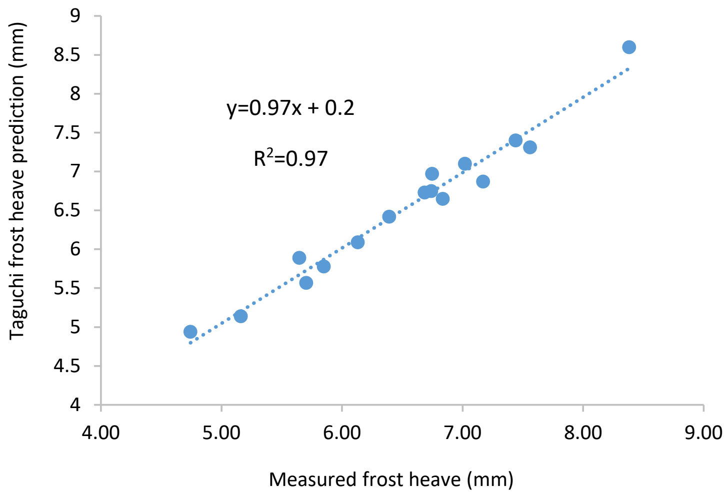

Fig. 5: Results of frost heaves measured versus Taguchi predictions

the Taguchi predictions were used afterward.

$$R = \frac{\Sigma\left[(X - X_m)\times(Y - Y_m)\right]}{\sqrt{[\Sigma(X - X_m)^2 \times \Sigma(Y - Y_m)^2]}} \quad (13)$$

A test was conducted for 10^8 CFU/mL bacteria concentration, 21 days of curing, and 0.6 CSC to justify the optimum predicted condition. Accordingly, the frost heave was found as 4.41 mm and the heave ratio was obtained as 0.21. These results were completely in agreement with the Taguchi predictions.

Heave ratio diagrams

Three diagrams for each input factor at its optimum level are shown in Fig. 6. The heave ratio of various bacteria concentrations and curing times were plotted for a CSC of 0.6 M (Fig. 6a). The same diagrams were delineated for 21 days of curing time and 10^8 CFU/mL of bacteria concentration (Figs. 6a and b). The heave ratio was in the range of 0.21-0.42, depending on the CSC, bacteria concentration, and curing time. This implied that the MICP treatment significantly decreased the amount of soil frost heave. Even the minimum treatment with no curing led to a 42% reduction in the soil frost heave. Therefore, the MICP treatment technique could be suggested as a viable alternative for the mitigation of the soil frost heave. As shown in Fig. 6, the amount of frost heave is changed with the variation of the CSC for the whole tests. However, the lowest frost heave was observed in the testing samples with 0.6 M CSC, after which the amount of the frost heave increased with the increase of CSC. Based on the testing results, the curing time of the treated soil samples had a significant role in their frost heave responses. The specimens with long curing periods presented less frost heave amounts in all the cases. The minimum frost heaves were observed in the samples treated with a CSC of

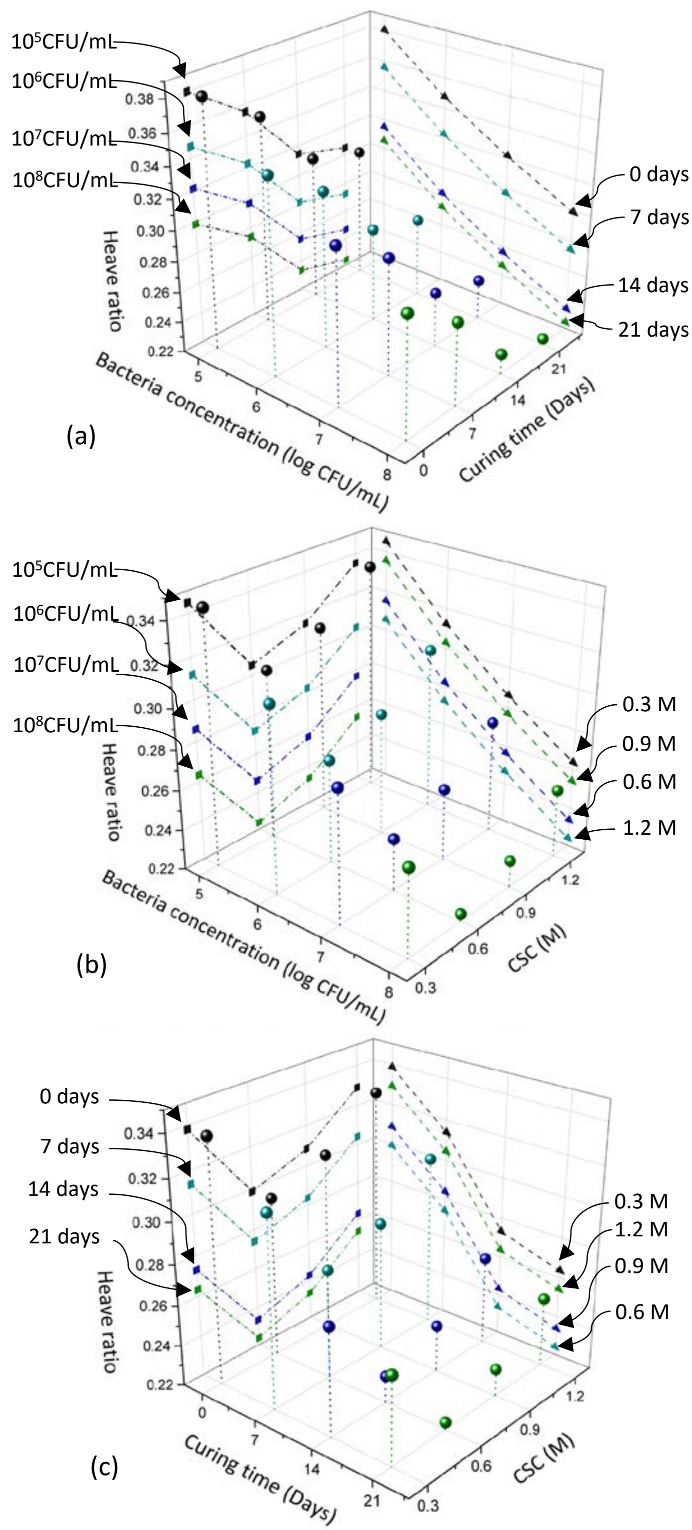

Fig. 6: Diagrams of heave ratios at 0.6 M CSC (a), 21 days curing time (b) and 10^8 CFU/mL bacteria concentration (c)

0.6 M and cured for 21 days for all testing bacteria concentrations.

Reduction of the MICP effect with the increment of CSC could be explained by the negative impact of a high salinity environment on the life and activity of the bacteria (Ng *et al.*, 2012). Higher carbonate precipitation causes lower soil frost heave due to the stronger bound among the particles and filling the voids among the soil particles, resulting in less water preservation within them (Lambe, 1956). As can be seen in Table 5, the bacteria concentration and curing time had a more significant role in the reduction of the soil frost heave compared to the cementation solution concentration. Notably, applying more bacteria concentrations imposed higher costs on the project, while curing the treated soil required very lesser expenses and attention.

Comparison of the MICP with previous methods

Numerous researchers have focused on the soil frost heave treatment. For instance, Lambe *et al.*, (1956) examined the effect of 40 additives in the reduction of frost heave. Kettle and McCabe, (1985) used the mechanical soil improvement method by mixing slag, basalt and limestone with soil. They reported 0.24 to 0.41 heave ratios for different materials in different sizes. Arabi and Wild, (1986) used lime to control the soil frost heave. They reported an increase in the frost heave level for using 2% or less lime, but the frost heave decreased as expected in higher percentages of lime and higher temperature and curing time. Guthrie *et al.* (2007) employed cement to control the frost heave, and reached higher amounts of frost heave for the soils treated with 2% or less cement rather than the untreated soil samples. They also demonstrated that the frost heave dramatically decreased when 3.5% or more cement additive was employed. Hu *et al.* (2018) used nano-silica to reduce the soil frost heave. They observed a slight reduction in frost heave (about 3.6%) when the nano-silica content was less than 1% or more than 2%. The claimed that the frost heave reduction was about 26% (0.74 heave ratio) when the applied nano-silica content was in the range of 1%-2%. Zieba *et al.* (2019) used 5% of nano-silica content to reduce the soil frost heave. They reported 97% (0.03 heave ratio) reduction in the frost heave for the treated sample. They also used 5% micro-silica which resulted in 21% (0.79 heave ratio) reduction in frost heave. Velsovskij *et al.* (2020) employed the anti-icing materials which resulted in 30% to 33% (0.67 to 0.70 heave ratios) reduction in frost heave. The results of a few previous studies are presented in Fig. 8 for comparison. These results are illustrated based on the heave ratios of the used soil (0 corresponds to a zero frost heave). Comparison of the results from Fig. 8

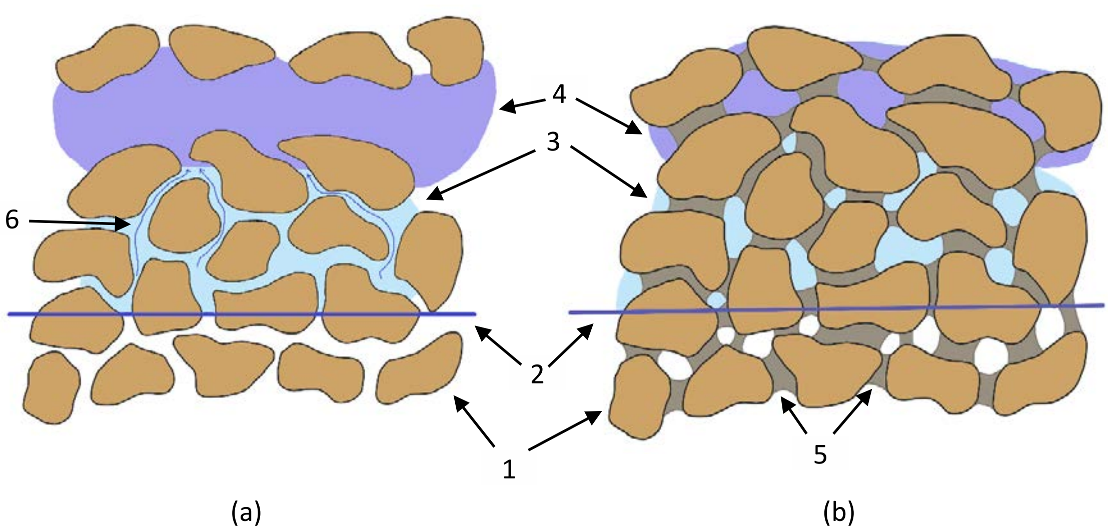

Fig. 7: Frost heave of untreated (a), and treated (b) soils: soil particles (1), freezing front (2), void ice (3), ice lens (4), precipitated calcite (5), and the path of water through ice lens (6)

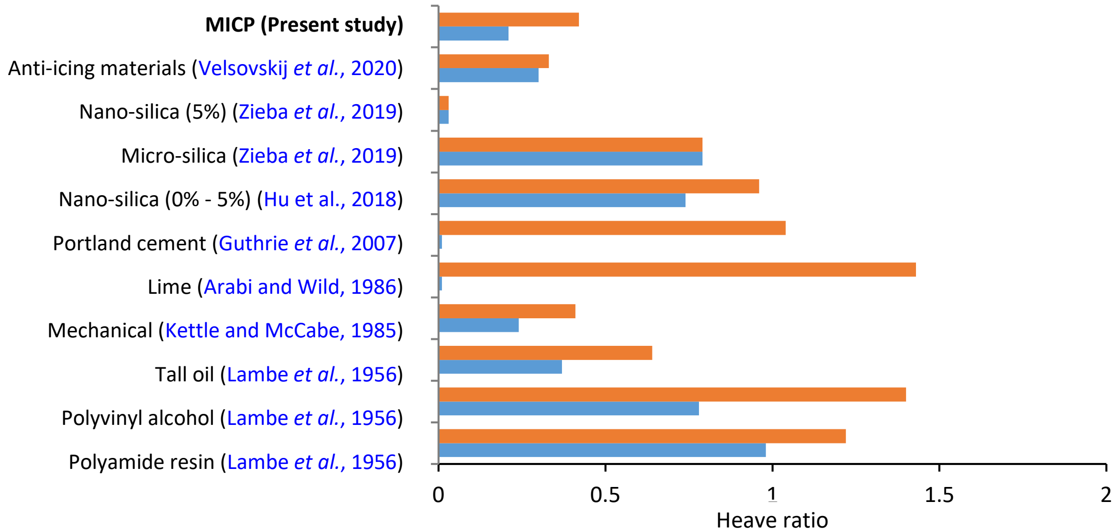

Fig. 8: Comparison of the different methods for the mitigation of frost heave

showed that the MICP used for controlling the soil frost heave let to a reasonable outcome compared to other methods. For example, higher amounts of lime or cement should be used to reach an acceptable outcome; otherwise, these additives would end up with adverse effects. Compared to other methods, the heave ratio of the samples treated with the MICP method was acceptable, showing the technical feasibility of the MICP method to confront the soil frost heave. This method can also eliminate the environmental pollution using the toxic synthetic materials.

The heave ratios of the MICP-treated samples were in the range of 0.21 to 0.42. The obtained heave ratios were smaller than the ratios of polyamide resin, polyvinyl alcohol, nano-silica (0%-5%), micro-silica, Portland cement (less than 2% content), and lime (less than 3.5 % content, or not cured at high temperatures). The Portland cement and lime (cured at 75 °C) showed a better function when utilized in more than 2% and 3.5% contents, respectively. The curing of lime at 75 °C was somehow impractical to some extent. Application of nano-silica (5%) mitigated the soil frost heave almost completely. Considering the debates over the possible health issues of nano-silica, some researchers believe that it causes chronic diseases like silicosis (Napierska *et al.*, 2010). Mechanical stabilization methods led to 0.24 to 0.41 heave ratios. This range was approximately the same as the range obtained in the MICP method. This method can be utilized for shallow soil improvement, while using it for deep layers is challenging, time-consuming, and costly. The MICP and other methods, which inject grouts to improve the soil characteristics, have the advantage of being employed in deeper layers with no necessity for removing upper levels. The obtained heave ratio for anti-icing materials was 0.3-0.33 which is nearly the same as the ratio obtained in the MICP method. The used materials were saline-based materials which reduced the water freezing point.

MICP efficiency justification through scanning electron microscopy (SEM) images

The images of both untreated and MICP-treated samples were provided by the SEM technique. For SEM imaging, a small specimen was carefully prepared with the given characteristics required for such imaging techniques. In this context, for both treated and untreated soils, a cubic representative specimen with a side length of 25 mm was separated from bulk samples with strict care. The SEM images of the treated specimen, from a typical testing case alongside those of the untreated soil specimens, are presented in Fig. 9. The quality and magnitude of calcite precipitation on the soil particle surfaces within the soil matrix can be evaluated by looking at the SEM, particularly at magnified spots of 1 and 2.

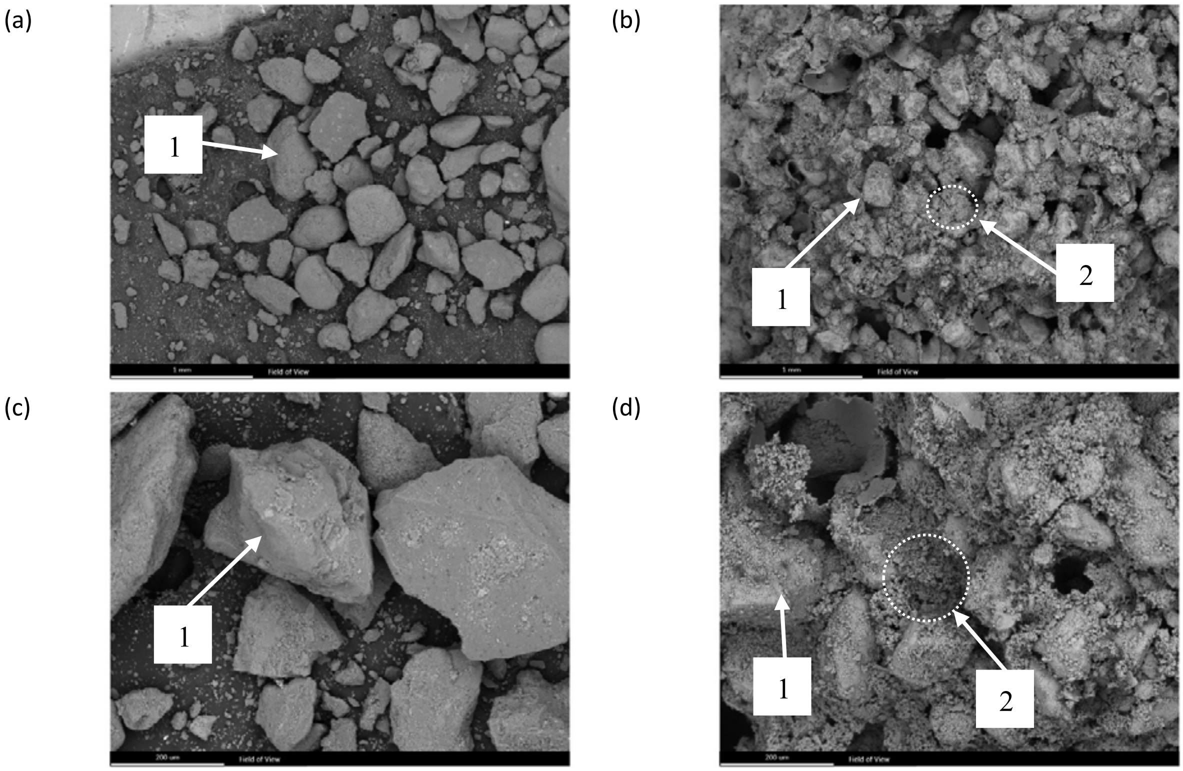

Fig. 9: SEM images of untreated (a,c), and treated T14 (b,d) soil samples (the magnification of each image is illustrated at its left bottom corner)

These images are similar to those illustrated in the previously reported studies on the MICP treated soils (DeJong *et al.*, 2006). Zamani and Montoya, (2018) illustrated the occurrence of calcite precipitation on the soil grains and the clear difference between the texture of the treated and untreated soils. As shown in Fig. 9b and d, the uniform formation of calcite crystals was observed on the surface of soil particles and also within the pores among the soil particles. Although the calcite formation was not too great to completely fulfill the soil pores, nonetheless properly bonded the soil particles and presented a robust and less permeable soil matrix. The partial fulfillment of pores with calcite led to a lower saturation degree in the capillarity zone which lowered the soil frost heave.

CONCLUSION

The soil frost heave is a common problem in cold regions and mainly destroys the infrastructures in the suburban areas such as roads, railways, and pavements. In this study, a novel approach was proposed to overcome this issue. For this purpose, a laboratory-scale experimental study was carried out to assess the efficiency of the MICP bacterial treatment to mitigate the frost heave of typical silty sand. An innovative frost heave testing apparatus was designed to meet the requirements of the soil frost heave measurement and control. The *Sporosarcina pasteurii* bacterium was employed to proceed with the MICP procedures for soil treatment. The MICP method was introduced as a viable and efficient alternative to conventional methods for treating the soil frost heave for the first time. According to the results, the *Sporosarcina Pasteurii* bacterial MICP treatment led to a reduction in the frost heave ratio to 0.21 in optimum treatment (i.e., lowering the soil frost heave to nearly one-fifth of the untreated soil). The minimum MICP treatment showed a heave ratio of 0.42. The ANOM and ANOVA analyses of data developed by the Taguchi-based experimental design showed that, among the three variables of the bacteria concentration, curing time, and CSC, the bacteria concentration with 41.69% contribution to the results was the most influential factor. The curing time had a 40.75% contribution to the outcome, which was slightly less than the contribution of the bacteria concentration factor. The lowest contribution belonged to CSC, with 12.90% contribution. The P-values for all the three factors were less than 0.05, and thus the null hypothesis was rejected. The frost heave trend assessment revealed that increasing the bacteria concentration continuously decreased the soil frost heave. The analysis also defined the optimum (minimum) frost heave occurring at 10^8 CFU/mL bacteria concentration (the maximum level). The curing time factor also followed the same trend, and the minimum frost heave occurred at 21 days, (the highest curing time). The optimum CSC, leading to the lowest frost heave, was found to be 0.6 M. However, in contrast to the other two factors, the frost heave increased to higher levels of 0.9 M and 1.2 M. Except for using over 2% of cement, in application of 3.5% of lime cured at 75 °C, and 5% of nano-silica (which reduced the heave ratio to near zero), the heave ratios of the MICP-treated samples were lower. In a few cases, their range was the same in different methods. In contrast to other methods namely cement, lime, and nano-silica, one of the advantages of the used method was its effectiveness at any application level. The observational SEM imaging approved the formation of calcite precipitation, bonding soil particles, and filling soil pores. Therefore, the partial filling of soil pores, due to the MICP procedure, led to a lower saturation degree in the capillarity zone which lowered the soil frost heave. The MICP method faces some challenges, namely providing some undesirable by-products. This method may be used as an eco-friendly, cost-effective approach after development, modification, and industrialization in the future.

AUTHOR CONTRIBUTIONS

M.F. Nikshoar contributed to the literature review, experimental design, performing laboratory tests, data analysis, and writing the original draft. M.A. Rowshanzamir supervised the geotechnical laboratory tests, methodology, data analysis, writing review, and editing. S.M. Abtahi is the paper's corresponding author and performed conceptualization, methodology, and data compilation. S. Soleimanian-Zad supervised the biological laboratory tests and activities, performed biological methodology and biological investigation, writing reviews on biological aspects.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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ABBREVIATIONS

%	Percent
°C	Degree Celsius
ANOVA	Analysis of variance
ATCC	American Type Culture Collection

ANOM	Analysis of means
ASTM	American Society for Testing and Materials
BC	*Bacteria concentration*
Ca^{2+}	Calcium ion
$CaCO_3$	Calcium carbonate
Cc	The coefficient of gradation
CFU	Colony-forming unit
cm	Centimeter
cm^3	Cubic centimeter
CO_2	Carbon dioxide
CP	Contribution percent
CP_{er}	Contribution percent of errors
CSC	Cementing solution concentration
Cu	The uniformity coefficient
D_{50}	The median particle diameter
DOF	Degree of freedom
Eq.	Equation
Exp.	Experiment
FH	Frost heave test result
$\overline{FH}$	Mean frost heave
Fig.	Figure
g	Gram
g/L	Grams per liter
g/M	Grams per molar
G_s	The specific gravity of soil
H_2O	Water
HCO_3^-	Bicarbonate ion
Heave ratio	Average heave of treated soil divided by the average heave of untreated soil
hour	Hour
i	Number of the test repetition
IUT	Isfahan University of Technology
KN	Kilonewton
L	Liter
log	Logarithm
m	Number of experiments
M	One mole per litre
m^3	Cubic meter
MICP	Microbially induced calcium carbonate precipitation
min	Minute
mL	Milliliter
mm	Millimeter
MS	Mean of squares
n	Total number of repetitions for that factor
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
$(NH_2)_2CO$	Urea
NH_3	Ammonia
NH_4^+	Ammonia ion
NH_4Cl	Ammonium chloride
No.	Number
OD_{600}	Optical density of the biomass measured at 600 nm wavelength
OH^-	Hydroxide
p-value	Probability value
PTFE	Polytetrafluoroethylene
R^2	
rpm	
saclSi	Sandy clayey silt
SEM	Scanning Electron Microscopy
SM	Silty sand
SNR	Signal-to-noise ratio
SOP	Significance of the parameter
SS	
SS_T	
t	Level number if the test
TDOE	Taguchi's design of experiment
USB	Universal Serial Bus
X	Data points in the X data series
X_i	The i data point
V_{er}	Variance of error
$\bar{X}_T$	Total average of the data points
$\bar{X}_z^f$	Average value of the outputs for the parameter f
X_m	The mean of data points in the X data series
Y	Data points in the Y data series
Y_m	The mean of data points in the Y data series
$\gamma d\ min$	The minimum density of soil
$\gamma d\ max$	The maximum density of soil
Z	Number of levels for each input factor

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