Engineering Properties of Sandy soil Improvement with Bacillus Simplex

Baki Bagriacik (bakibagriacik@gmail.com)
Cukurova University: Cukurova Universitesi https://orcid.org/0000-0002-1860-2881
Zahrettin Kabir Sani
Cukurova Universitesi
Fatima Masume Uslu
Cukurova Universitesi
Esra Sunduz Yigittekin
Cukurova Universitesi
Sadik Dincer
Cukurova Universitesi

Research Article

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Abstract

Purpose: Stabilization of weak soil can be achieved through different methods, some of which include: jet column, cement stabilization and fly ash stabilization. Unfortunately, the use of the aforementioned methods of soil improvement affects the environment negatively thereby leading to environmental degradation. With the aforesaid impediment in mind, the need for devising methods of weak soil improvement becomes pertinent.

Methods: *Bacillus sp*. - a non-pathogenic organism found abundantly in soil - was investigated in this study as a potential agent of soil improvement. The usability of *Bacillus sp.* in soil improvement was investigated with direct shear tests and permeability tests under optimum conditions in this study.

Result: Time-dependent study on the effect of the ureolytic bacteria *Bacillus simplex* induced calcium carbonate precipitation shows reduction in permeability and increase in the strength of the soil under study. On exhaustion of the available nutrients in the soil however, the strength of the soil is not negatively impacted.

Conclusion: Microbially induced calcium precipitation by *Bacillus sp*. is effective in soil improvement as such it may serve as substitute for conventional soil stabilisation techniques. The ability of the bacteria to precipitate calcium carbonate in the soil leads to reduction in the permeability and increase in the shear strength of the soil.

Background

Numerous studies have been conducted to assess the effect of microbially calcium carbonate precipitation on the permeability and strength of poor soils. Changes in compressibility, permeability and strength of the treated soil depend on numerous environmental conditions which interfere with microbial response towards specified reagents by so doing affecting their ability to precipitate calcite. For the past few years, utilizing the ability of bacteria to precipitate calcium carbonate ($CaCO_3$) has been found to be a promising eco-friendly approach of soil improvement (DeJong et al., 2006). The evolvement of an alkaline microenvironment as a result of bacterial physiological activity leads to the deposition of calcium carbonate (Douglas and Beveridge, 1998).

Ureolytic bacteria had been employed in conducting Significant investigations on carbonate precipitation by bacteria (Stocks et al., 1999). Ureolytic bacteria enhance the precipitation of $CaCO_3$ through the production of the enzyme urease. Urease enzyme catalyses the hydrolysis of urea to CO_2 and ammonia thereby causing an increase in pH concentration and carbonate (Stocks et al., 1999). Bacterial precipitation of calcium carbonate has been found to elevate the bearing capacity of soil (Lo Bianco and Madonia, 2007; Dejong et al., 2006). Bacterial calcium carbonate precipitation has been utilised in the crack repair of granite and concrete (Gollapudi et al., 1995, Ramachandran et al. 2001; Bang et al. 2001; Ramakrishnan, 2007; Jonkers et al., 2009). These precipitations were found to fill pores, reduce permeability through the enhancement of particle bonding (Ivanov and Chu, 2008; Whiffin et al., 2007).

Previous researches have pointed the bacteria *Bacillus pasteurii* - which exhibits high urease production capability – as a potential candidate suitable for been utilised in biocementation (Bang et al., 2001; Dejong et al., 2010; Bachmeier et al., 2002, Sarda et al., 2009). The researches indicate that biocementation can serve as an effective technique in reducing soil permeability. Because of the damage that moisture causes to building foundations, the need arises for altering the permeability of the foundation soil. Despite the fact that numerous researches have been conducted with the aim of reducing soil permeability, there is limited research on the utilization of bacteria in this field. Ferris et al., (1997) and Whiffin et al., (2007) have observed that biocementation in sandy soil reduced permeability significantly. Nemati and Voordouw (2003) found that calcite cementation in sandstone reduced permeability by 98%. Biocementation arises due to microbial activities that lead to the production particulate binding materials thereby improving the soil structure (Ferris et al., 1997; Nemati and Voordouw, 2003; Whiffin et al., 2007). In addition to enhancing the shear strength of tropical soil, bacterial calcite precipitates have been reported to reduce the permeability of tropical soil, however, high salinity has an inhibitory effect on calcite precipitation by the bacteria (Soon et al. 2013-2014). 22 – 75% soil permeability reduction has been reported by Whiffin et al. (2007). On treating soil with the enzyme urease, Yasuhara et al. (2012) reported a permeability reduction of 60 - 70%. So also, inoculating soil with the bacteria *Bacillus megaterium* has been reported to induce a 90% reduction in hydraulic conductivity (Soon et al., 2014; Umar et al., 2016.

Calcite precipitation reduces the pore cavities of soil and by extension effecting permeability reduction (DeJong et al., 2010). Chu et al. (2012) studied shear strength reduction and hydraulic conductivity of soil using the ureolytic bacteria *Sporosarcina pasteurii* isolated from tropical coastline soil. They also found that the cracking modulus of the lean calcium carbonate layer formed on the soil surface was 35.9 MPa, described Microbially induced Calcium Precipitation has been described as a breakthrough technique of soil improvement (Filet et al. (2012.

Bio-mineralized calcium carbonate has been shown to be effective in bioassay of soils and can be used in geotechnical engineering (Ivanov and Chu, 2008). The investigators stressed that these strategies can serve as substitutes to conventional techniques which tend to be expensive and in some cases impact negatively on the environment. Yasuhara et al., (2012) utilized urease enzyme obtained from sources other than microbial in catalysing urea hydrolysis within the proximity of calcium chloride and found out that dramatical increase in strength and 60% reduction of hydraulic conductivity of the treated soil samples occurred. Canakci et al., (2015) investigated the effect of bacterial calcium carbonate precipitate on the compressibility and strength of organic soil and found that bacterial treatment influenced the compressibility and shear strength of the organic soil. Although, several researches utilizing *Bacillus* sp in sandy soil improvement have been conducted, significant studies investigating the effect of time-dependent *Bacillus* sp. treatment on the strength and permeability of poorly graded soils have not been conducted.

Results And Discussion

The particle size distribution of the sample was found to be 53.6% fine grain and 46.4% medium grain sand (Figure 2). The soil was found to have 26.80 kN/m^3 specific gravity, 15.03 kN/m^3 minimum dry specific gravity and 17.06 kN/m^3maximum dry specific gravity. Tabel 1 and 2 presents the physical and chemical properties of the soil sample under study.

Table 1

Soil sample physical properties

Granulometric Parameters	Unit	Value
Percentage of Medium Grained Sand	%	46.40
Percentage of Fine Grained Sand	%	53.60
Effective Grain Size, D_{10}	m	0.0018
D_{30}	m	0.0030
D_{60}	m	0.0050
Coefficient of Uniformity, C_u	-	2.78
Coefficient of Curvature, C_c	-	1.00
Soil Class	-	SP
Maximum Dry Specific Gravity	kN/m^3	17.06
Minimum Dry Specific Gravity	kN/m^3	15.03
Specific Gravity	kN/m^3	26.80

Table 2

Soil sample Chemical properties

Component	Sandy Soil (%)
SiO_2	96.15
Al_2O_3	2.10
Fe_2O	0.6
MgO	0.04
CaO	0.22
MnO	-
K_2O	0.39
Na_2O	0.07
TiO_2	-
P_2O_5	-

The bacteria isolated was identified as *Bacillus simplex* (Accession number: NR_1149191.1).

Soil shear strength

The internal friction angle and cohesion were determined based on the peak shear stress using Mohr-Coulomb failure criterion for all soil samples (Figure 3 – 5). The internal friction angle and cohesion coefficient of the treated soil were found to be 1.05 kPa, 38.51^0; 1.21 kPa, 38.93^0; 1.50 kPa, 40.19^0; 3.61 kPa, 42.90^0; 21.50 kPa, 42.30^0 and 43.10^0; 26.51 KPa respectively for the 1^{st}, 2^{nd}, 5^{th} 15^{th}, 30^{th} and 45^{th} day of inoculation. However, the internal friction angle and cohesion coefficient of the uninoculated control soil were found to averaged 1.05 KPa and 38.30^0 respectively for the whole period of the investigation which is in agreement with other researches (Zhang et al., 2006; Dafalla, 2013; Khaleghi and Rowshanzamir, 2019). Significant increase in shear strength of the soil sample treated with the isolated bacteria can be attributed to the cementation activity induced by the bacteria *Bacillus sp*. (Ramachandran et al., 2001; Rodriguez Navarro et al., 2003; Khaleghi and Rowshanzamir, 2019). Studies relating to the effect of curing time on soil treated with *Bacillus* sp. and that on the changes in strength and permeability of the soil after the production of calcium carbonate by the bacteria are limited. On inoculating the soil with *Bacillus* sp. cementing occurs and this leads to an increase in cohesion and internal friction angles of the soil, this increase is recorded within the first 15 days, after which no significant increase in internal friction angles was recorded.

On the 15th day, 3.44 times increase in cohesion was recorded. While on day 30 and 45 an increase of 20.5 and 25.27 times were recorded. The internal friction angle of the treated was found to increase by 1.12, 1.10 and 1.12 times on day 15, 30 and 45 respectively. The results indicate that the density and consistency of the treated soil increases during the first 15 days relative to increase in internal friction angle and cohesion effected by the cementation activity induced the inoculated bacteria.

When time-dependent analysis was conducted on the soil sample, it was observed that the increase in internal friction angle terminated on day 15 and the increase in cohesion continues on condition that the inoculated bacteria is supplied with nutrients. On not supplying the bacteria with nutrients increase in cohesion property of the soil stops as soon as the available nutrient in it becomes exhausted by the bacteria and this does not lead to reduction in strength of the soil (Figure 6).

Soil permeability

Permeability coefficient of the soil inoculated with *Bacillus* sp. was observed to plummet by 30.36 times after 45 days (Figure 7), this can be attributed to the increase over time in the amount of calcium carbonate produced by the bacteria. Calcium carbonate produced in the soil reduces the gaps existing between soil particles thereby enhancing the resistivity of the soil against volume changes and this manifests evidently in permeability decrease of the soil. Hydraulic permeability decrease leads to the formation of more stable structure which may withstand liquefaction in the event of an earthquake. On exhaustion of the available nutrients in the treated soil, reduction in permeability was not observed (Figure 8).

Scanning Electron Microscopy (SEM) and X-ray diffraction (XRD)

SEM investigation reveals the presence of calcium carbonate particles in soil treated with *Bacillus* sp, however, the investigation shows that unlike the treated soil, in the untreated control soil calcium carbonate was not present (Figure 9). The result of X-ray diffraction analysis (XRD) of both the treated and untreated soil is presented in table 10, it indicates that ratio of calcium carbonate increases considerably in the soil treated with *Bacillus simplex* and this leads to the alteration in the chemical structure of the soil.

Conclusion

Stabilization of sandy soil had been achieved with the aid of the ureolytic bacteria Bacillus simplex. The ability of the bacteria to precipitate calcium carbonate in the soil leads to reduction in the permeability and increase in the shear strength of the soil. So also, the study shows that exhaustion of the available

nutrients in the improved soil do not impact negatively on the strength of the soil. In contrast with conventional techniques of soil improvement, this technique proves to be cost effective and eco-friendly. More studies should be executed in the future on the effect of calcium carbonate precipitation by the bacteria in-situ.

Methods

Physical and chemical characterization of the Soil

Sandy soil samples were collected from the riverbed of Seyhan river (37^{o}05’37.3”N 35, 35^{o}12’31.7”E) Adana, Turkey and were oven-dried. Physico-chemical characterization of the collected samples were conducted according to ASTM D 6913-04 established protocols.

Isolation and molecular characterization of *Bacillus simplex*

Urea hydrolysing bacteria was isolated from the rhizosphere soil of *Thuja orientalis* and *Pinus pinea* trees. Soil samples were collected from the rhizosphere of the aforesaid plants and 2 gram from each of the samples was homogenized in 10ml serum physiologic buffer, the resultant suspension was incubated at 85°C for 15 min with the aim of eliminating non-spore forming bacteria. 100 µl of the heat-treated sample suspension was spread plated on urea agar and incubated at 37 ° C for 24 hours.

The media for the experimental study was prepared by dissolving: 3g nutrient broth powder, 20g urea, 10g NH_4Cl 10gL^{-1}, 2.12g $NaHCO_3$ in 1 litre of distilled water, the pH of the solution was adjusted to 6.0 and autoclaved at 120^{o}C for 15min, 20ml of calcium chloride solution ($CaCl_2$. $2H_2O$ 18.5 g/100 mL) was added to the autoclaved medium (Canakci et al. 2015).

The identity of the isolated bacteria was determined through the amplification and sequencing of the its 16s rRNA gene. The 16sRNA gene sequence of the bacteria were aligned with known sequences using BLAST algorithm in the NCBI database. The bacterial gene sequences were deposited in the database and accession numbers were acquired.

Direct Shear Test

Direct shear test was conducted on the collected soil sample using the apparatus depicted in figure 1 to determine the shear properties, cohesion and internal friction angle (f) of the soil. The apparatus consists of a soil shear box, a loading head, a weight hanger and weights to generate normal loads. The shear box has two square rings for holding soil sample. The box has a cross-section of 60x60 and a height of 50.8mm. Horizontal displacement of the movable ring is achieved with a motor. A load cell measures the shear force. Two potentiometers measure the horizontal and vertical displacements. Values of shear

force and horizontal displacement is recorded by a computer data acquisition system (Sadek et al., 2011). The test was conducted using ASTM 3080 standards.

Bacillus simplex was inoculated into the soil sample. The inoculated soil was dried in an oven fed. on the 1^{st}, 2^{nd}, 5^{th}, 15^{th}, 30^{th} and 45^{th} day of inoculation, cohesion (c) and internal friction angle (f) of the soil were determined. Uninoculated soil sample served as a control.

Permeability Test

The permeability of the soil sample under analysis was determined in accordance with ASTM D2434-19 standard (Figure 2). Readings were taken throughout the permeability test at different time intervals and permeability coefficients determined.

Declarations

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Availability of data and materials:

The authors declare that all materials and data are available upon request.

Conflict of interests:

The authors declare that they have no conflict of interest.

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Authors' contributions:

Baki Bagriacik: Conducting experiments, Article writing, Literature search and writing, Evaluation of results, Review of article language.

Zahrettin Kabir Sani: Literature search and writing, article language review.

Fatima Masume Uslu: Isolation and characterization of Bacillus simplex

Esra Sunduz Yigittekin: Isolation and characterization of Bacillus simplex

Sadik Dincer: Article writing, Evaluation of results, article language review.

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Not applicable.

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Figures

Figure 1

Experimental setup

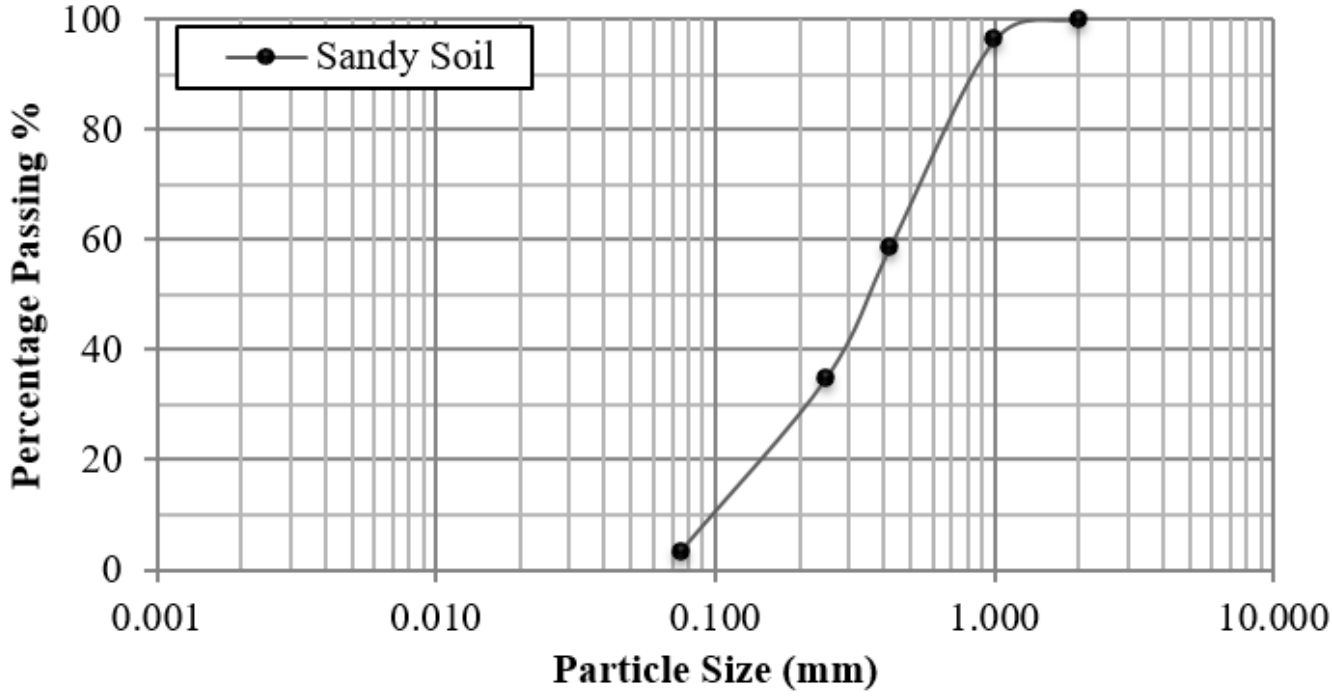


Figure 2

Sieve Analysis of soil sample

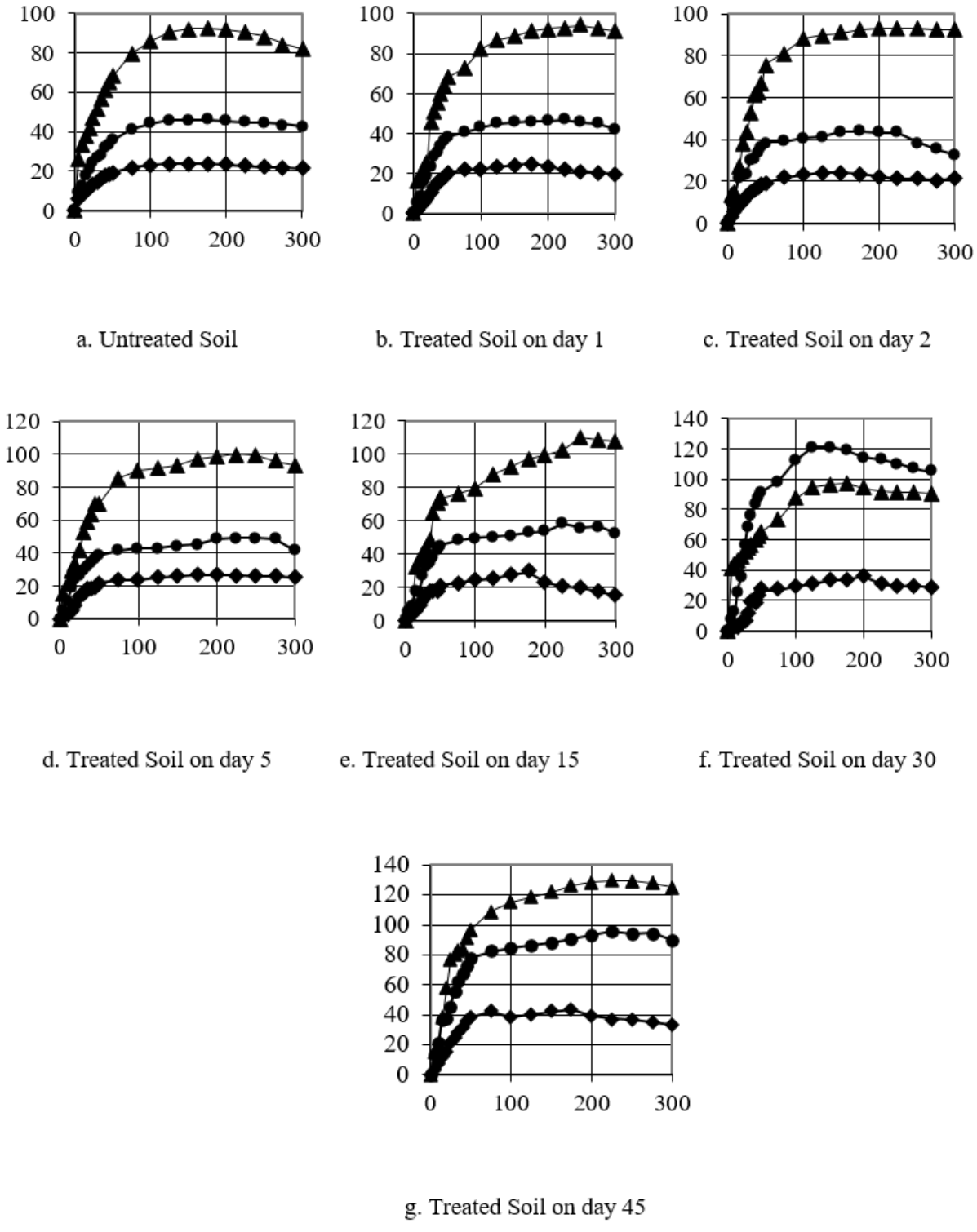


Figure 3

The Shear Stress-Horizontal Deformations of untreated and treated soil sample X axis: Horizontal Deformation (mm x 1/10), Y axis: Shear Stress (kN/m²)

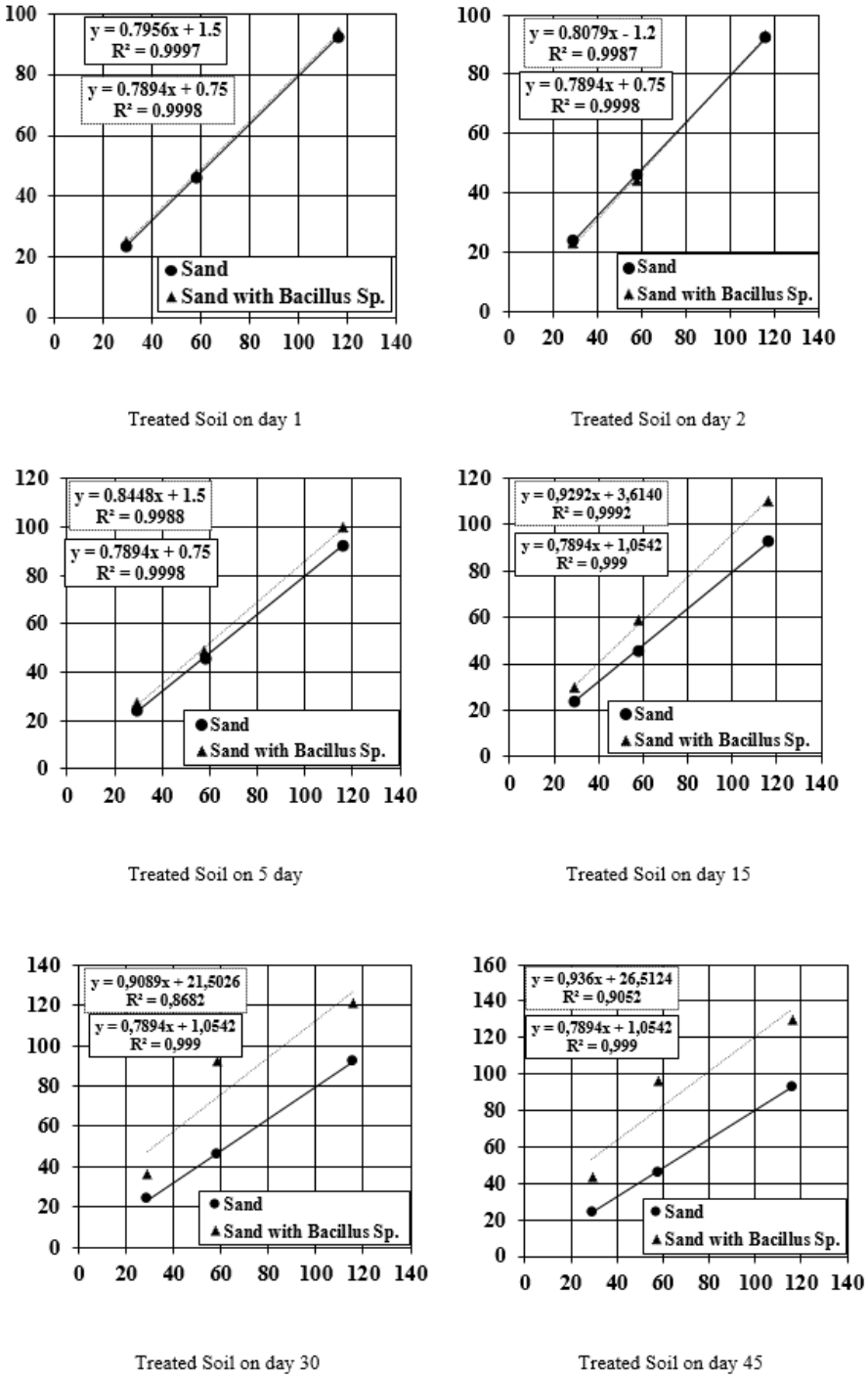


Figure 4

The Normal Stress - Shear Stress of untreated and treated soil samples

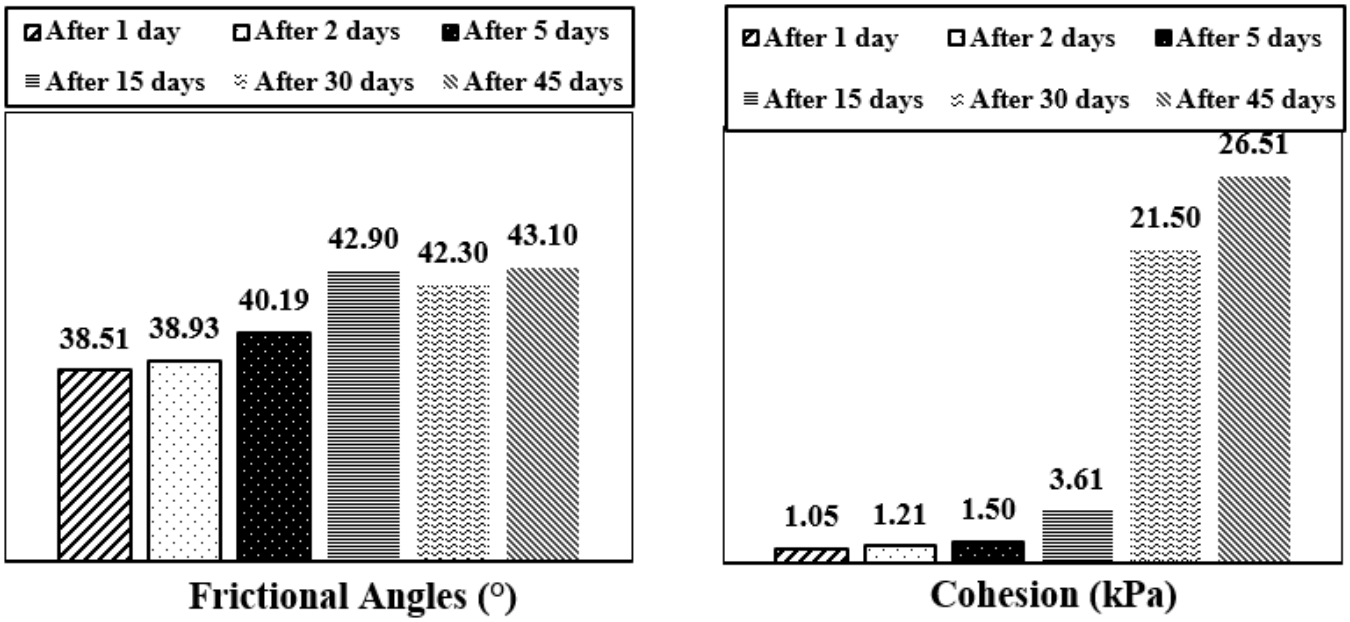


Figure 5

The frictional angle-cohesion values for all days

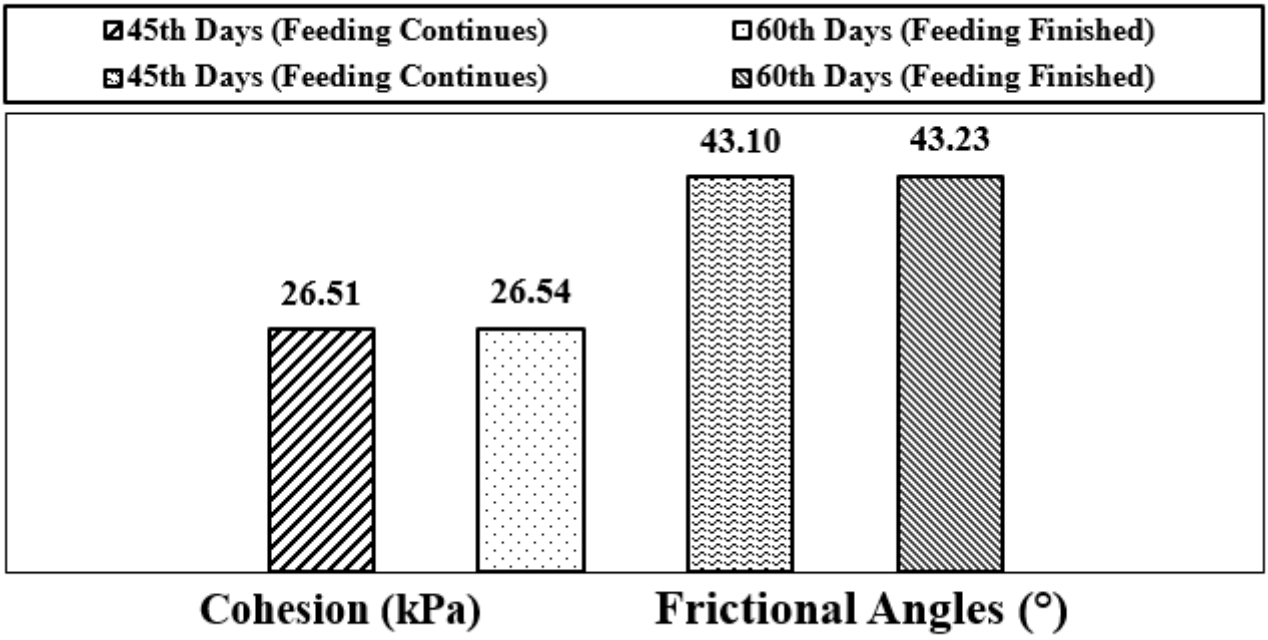


Figure 6

Sandy soil parameters during and after the completion of feeding

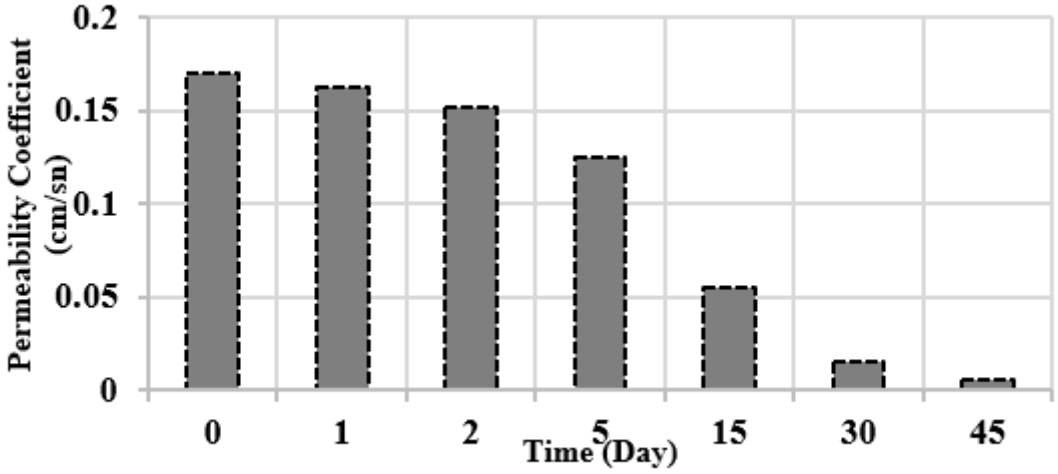


Figure 7

Permeability coefficient of the treated soil

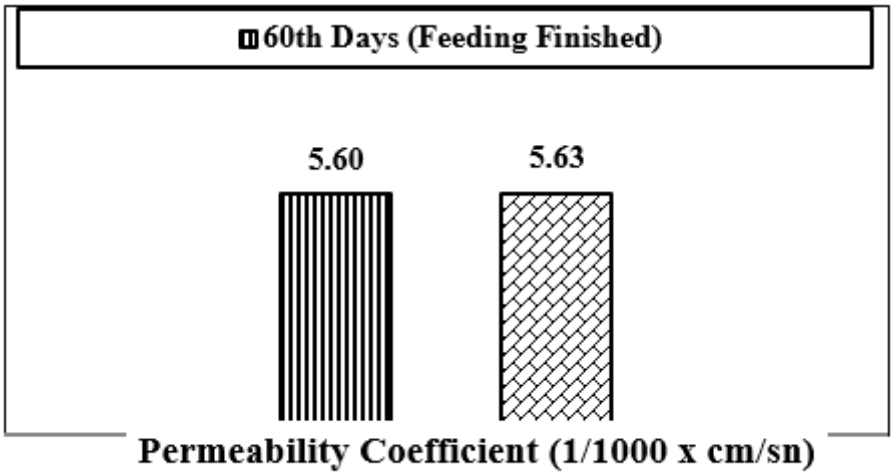
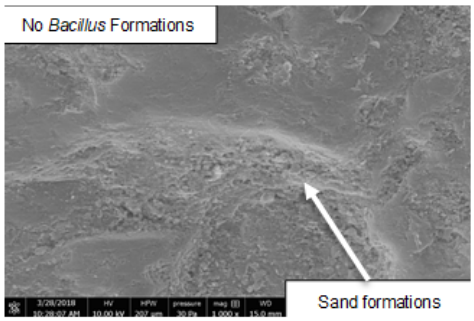
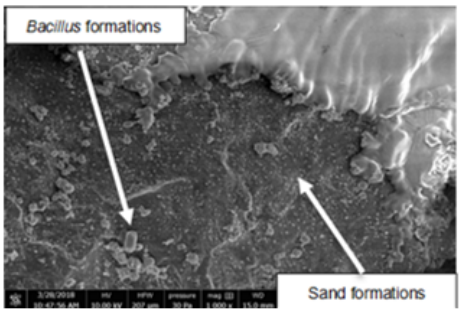


Figure 8

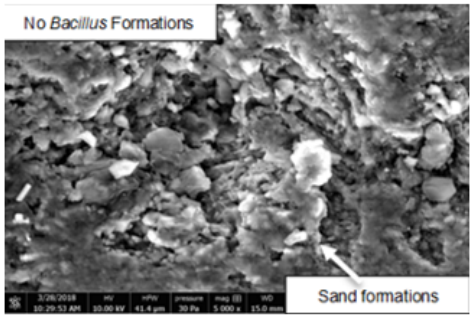
Permeability coefficient during and after the completion of nutrient supply



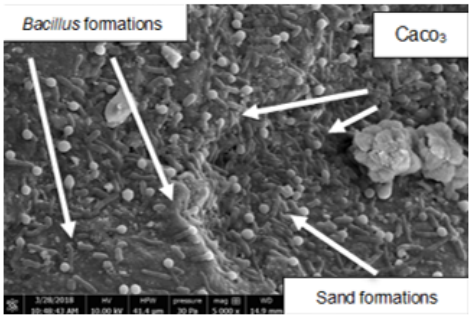
a. Sandy soil for 50µm



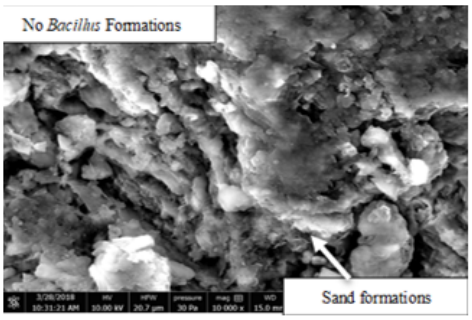
b. Sandy soil with *Bacillus* sp. for 50µm



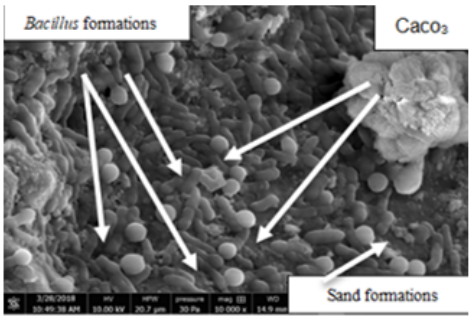
c. Sandy soil for 5µm



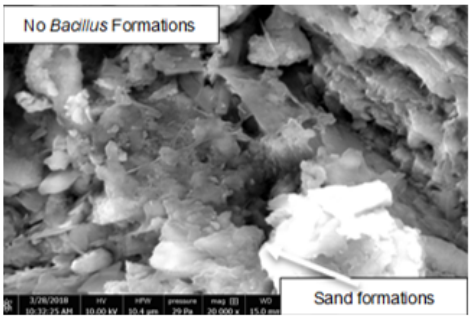
d. Sandy soil with *Bacillus* sp. for 5µm



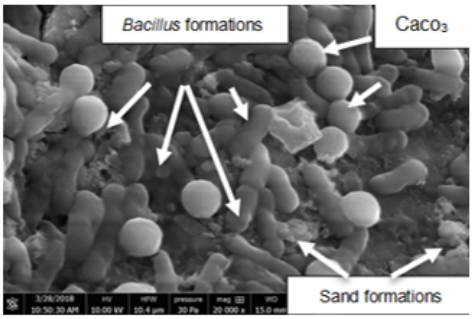
e. Sandy soil for 4µm



f. Sandy soil with *Bacillus* sp. for 4µm



g. Sandy soil for 2µm



h. Sandy soil with *Bacillus* sp. for 2µm

Figure 9

The images of SEM before stabilization (a,c,e,g) and after stabilization with (b,d,f,h)

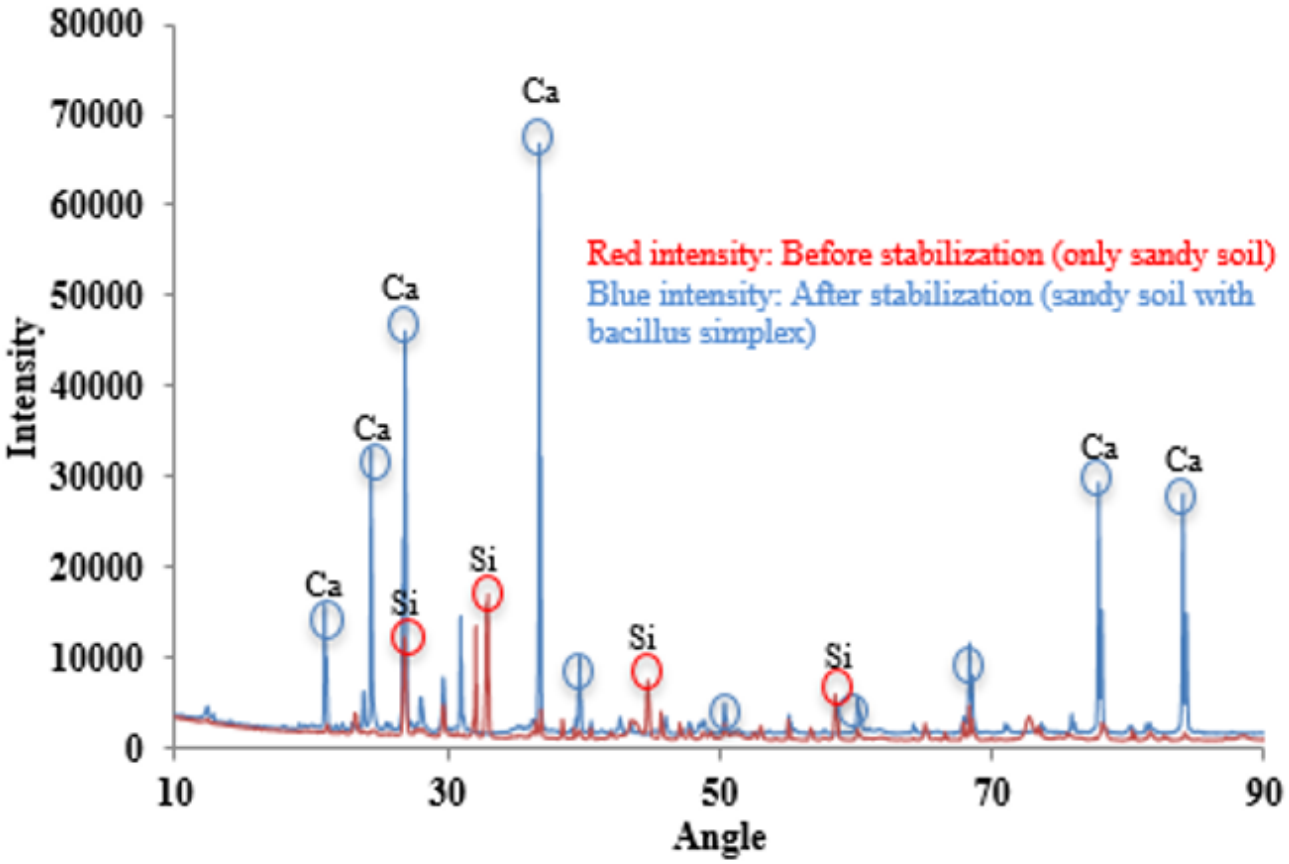


Figure 10

XRD analysis result of inoculated and uninoculated soil