



An experimental approach to microbial carbonate precipitation in improving the engineering properties of sandy soils

Baki Bagriacik^{1*} , Zahradden Kabir Sani², Fatima Masume Uslu², Esra Sunduz Yigittekin² and Sadik Dincer²

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 sp.*-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 stabilization 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.

Keywords: Soil improvement, *Bacillus sp.*, Time-dependent behaviour, Sustainability, Microbial carbonate precipitation, Sandy soil

Background

Numerous studies have been conducted to assess the effect of microbially precipitated calcium carbonate 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 specific reagents by so doing affecting their ability to precipitate calcite. Water accumulation in the soil can be a problem for civil engineering structures. When the load from the superstructure is transferred to the soil, there may be different settlements and swelling problems on the ground due to

the water in the soil. In addition, in such environments, depending on the soil type, there may be a risk of liquefaction during an earthquake. Thus, the accumulation of water on the soil damages the civil engineering structure. So low water permeability in the soil will prevent such problems. 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 involvement 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

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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 utilized 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 utilized 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 of particulate binding materials (calcite) 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). Whiffin et al. (2007) reported 22–75% soil permeability reduction. 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 and hydraulic conductivity of soil using the ureolytic bacteria *Sporosarcina pasteurii* isolated from tropical coastline soil. In the study, it was stated that cracks occur on the soil surface due to some conditions such as climatic conditions; these cracks were amended

with the produced calcium carbonate layers. Accordingly, the effect of calcium carbonate on the cracking modulus of the ground became stronger and was stated to be 35.9 MPa. Microbially induced calcium precipitation is stated to be a ground breaking technique in soil improvement (Filet et al. 2012).

Bio-mineralized calcium carbonate was found to be effective in increasing the bearing capacity of soil and reducing its permeability and settling. Ivanov and Chu (2008) by utilizing bio-mineralized calcium carbonate to improve the engineering properties of soil have shown that it can be used in geotechnical engineering. 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 in hydraulic conductivity of the treated soil occurred. Canakci et al. (2015) investigated the effect of bacterially induced calcium carbonate precipitate on the compressibility and strength of organic soil and found that bacterial treatment enhances 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. In the study conducted by Harianto et al. (2013), unconfined compression and permeability tests were carried out for different curing times on soils prepared using *Bacillus sp.* At the end of 28 days, an increase of 60% in the bearing capacity of the soil was observed. In addition, an 80% improvement in permeability values was observed at the end of 28 days. Therefore, 28 days were determined as the optimum curing time in terms of bearing capacity and permeability. In a study conducted by Hasriana et al. (2018), bearing capacity values were calculated for different *Bacillus sp.* ratios during a 7-day cure period. According to the results, it was found that as the amount of *Bacillus* increased, the bearing capacity increased up to 13 times. In another study conducted by Osinubi et al. (2019), bearing capacity values were calculated for samples prepared with different water content using *Bacillus sp.* The bearing capacity of soils with different water content was determined to be at most 278.0 kN/m² without *Bacillus sp.* This bearing capacity increased up to a maximum of 1835.5 kN/m² with the use of *Bacillus sp.* As a result, an increase in bearing strength of up to 6.6 times was observed. Studies on the use of *Bacillus* show that hydraulic permeability decreases by around 60% in a short time, and decreases up to 90% depending on time. In addition, it has been understood from the literature that there is an increase in the bearing capacity of soils from 6.6 to 13 times. It was seen that *Bacillus* is an alternative method in reducing the hydraulic permeability and increasing the bearing capacity of soils.

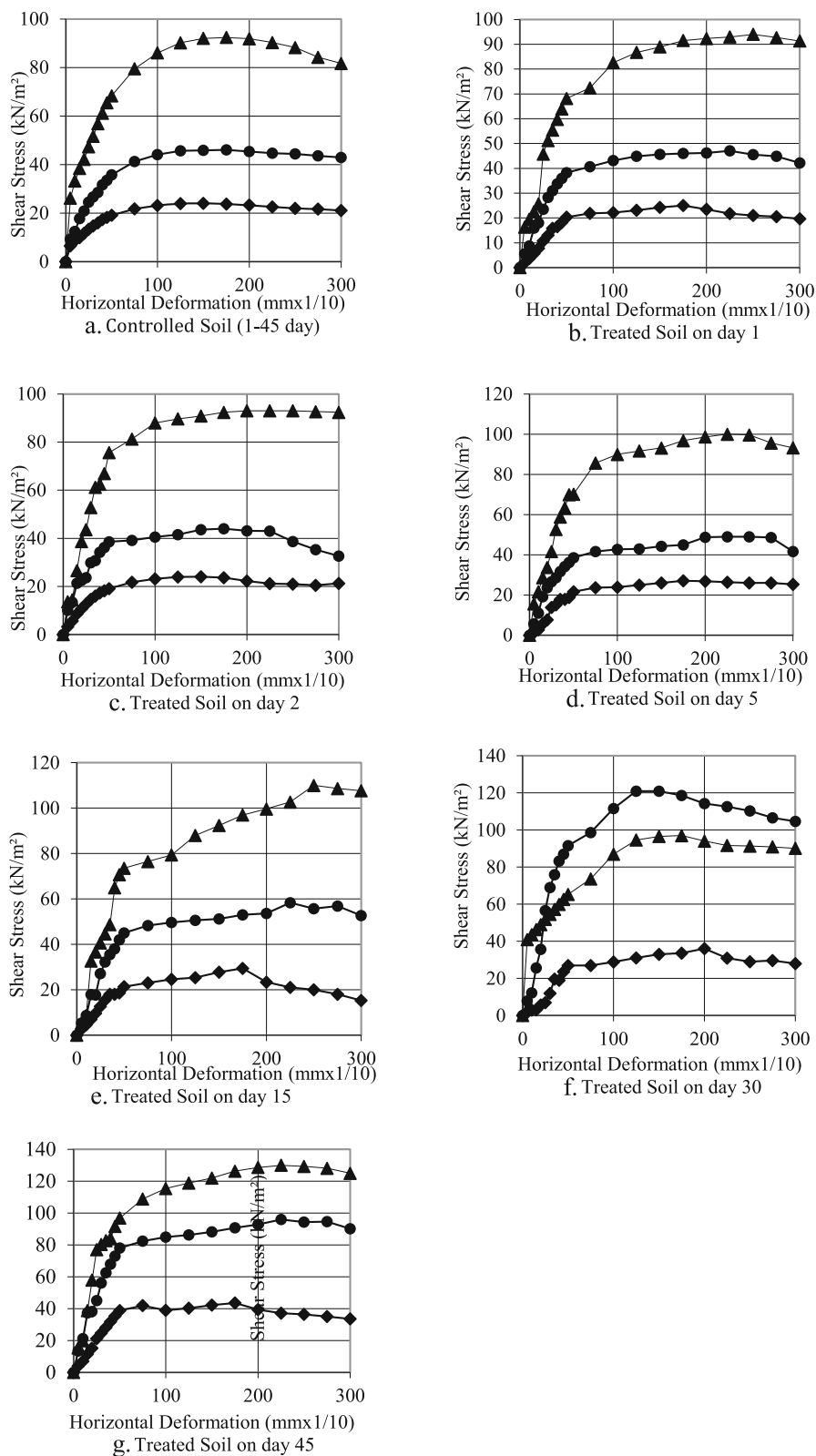
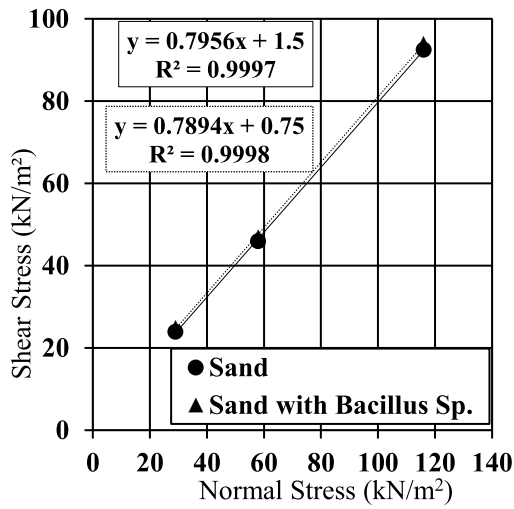
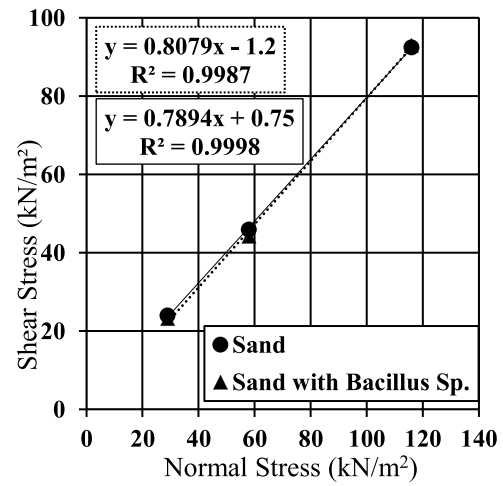


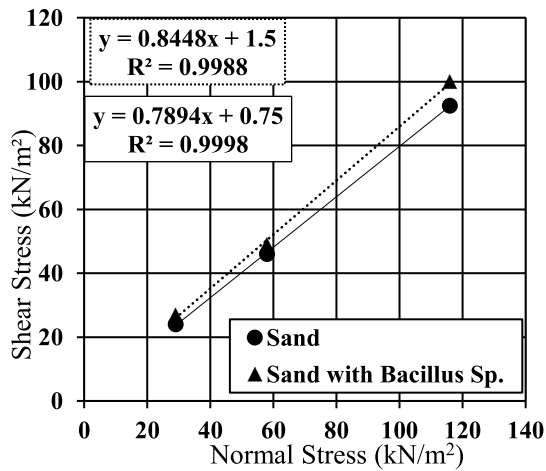
Fig. 1 The shear stress-horizontal deformations of controlled and treated soil sample



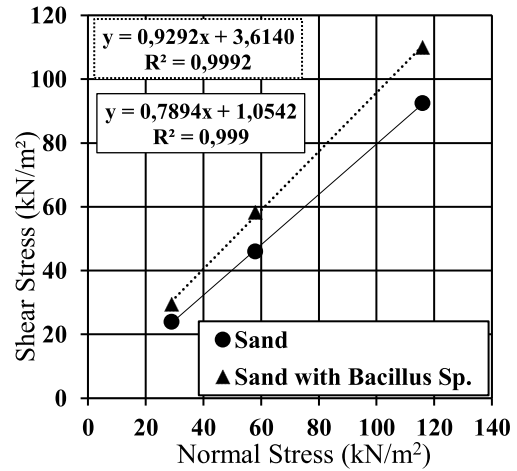
a. Treated Soil on day 1



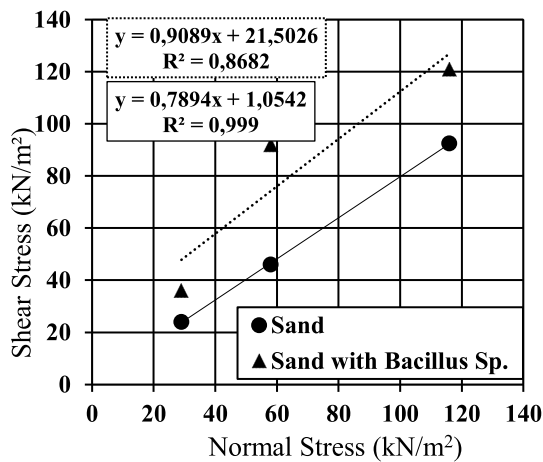
b. Treated Soil on day 2



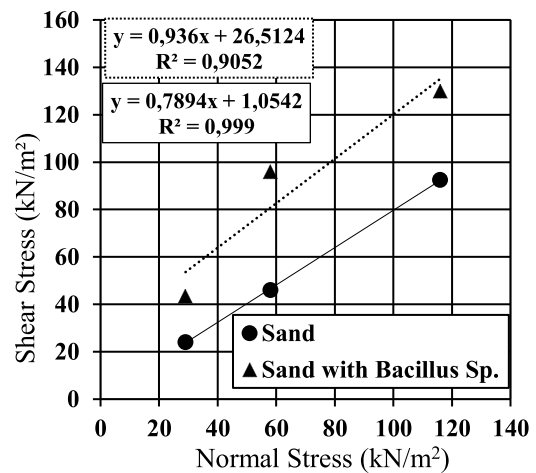
c. Treated Soil on 5 day



d. Treated Soil on day 15



e. Treated Soil on day 30



f. Treated Soil on day 45

Fig. 2 The normal stress-shear stress of controlled and treated soil samples

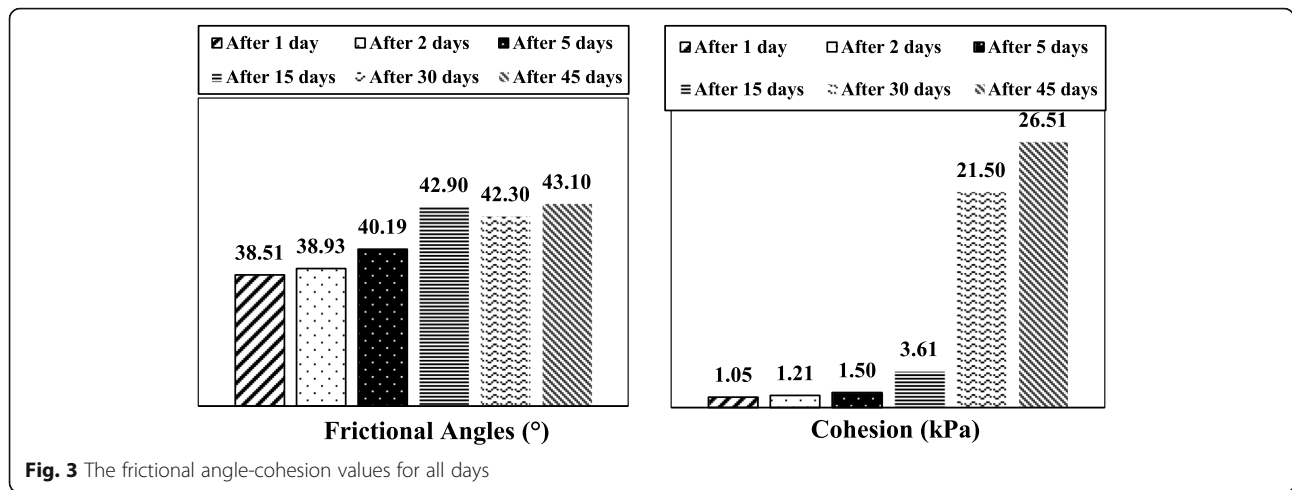


Fig. 3 The frictional angle-cohesion values for all days

When the literature was examined, it was seen that there were studies on the subject. However, due to the limited parameters investigated, this study was conducted to investigate different parameters (time-dependent strength and hydraulic permeability change, sustainability status, etc.).

Results and discussion

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 (Figs. 1, 2 and 3). The internal friction angle and cohesion coefficient of the treated soil were found to be 1.05 kPa, 38.51°; 1.21 kPa, 38.93°; 1.50 kPa, 40.19°; 3.61 kPa, 42.90° (Bagriacik et al., 2018); 21.50 kPa, 42.30°; and

43.10°; 26.51 KPa respectively for the 1st, 2nd, 5th 15th, 30th and 45th 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° (Bagriacik et al., 2018) 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

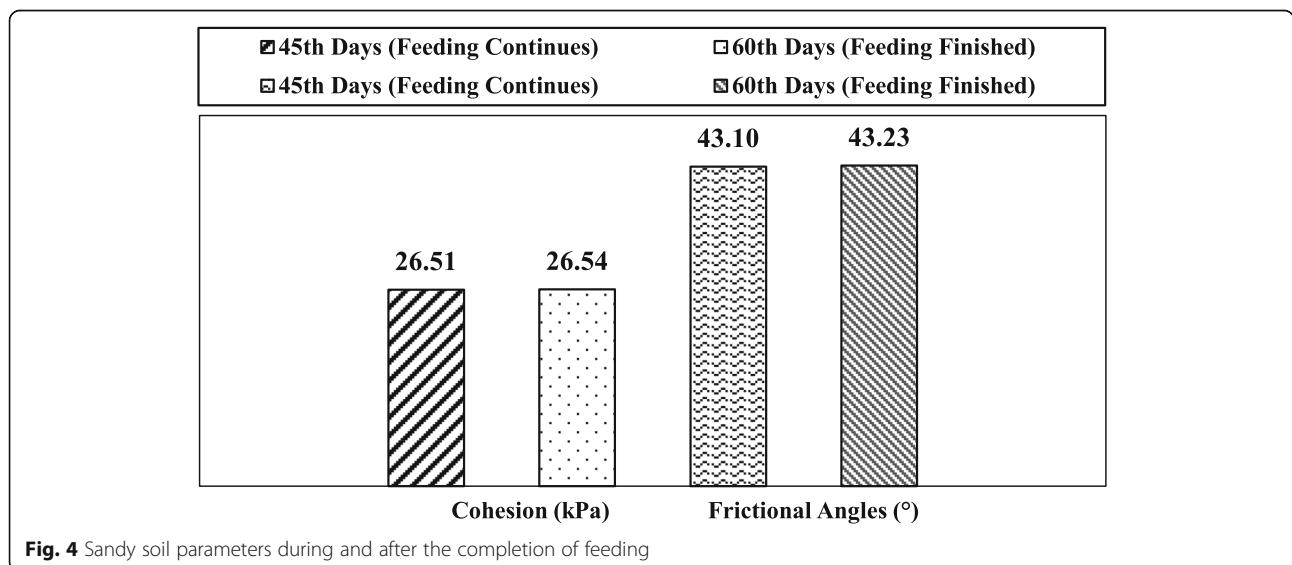
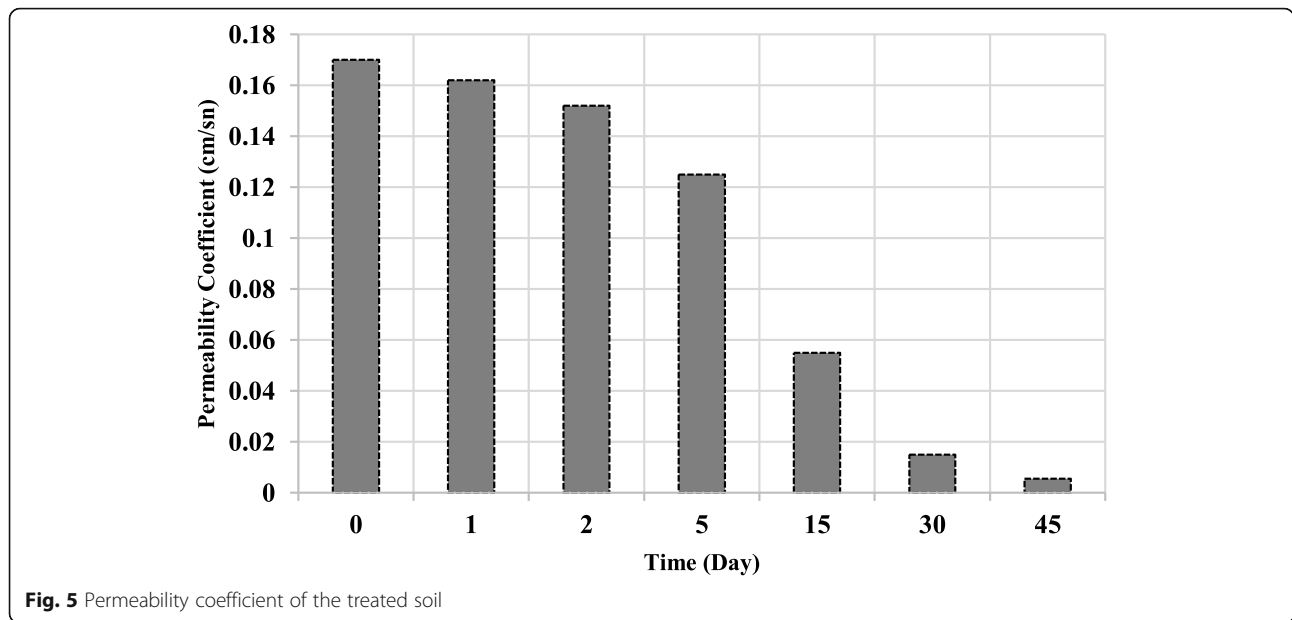


Fig. 4 Sandy soil parameters during and after the completion of feeding

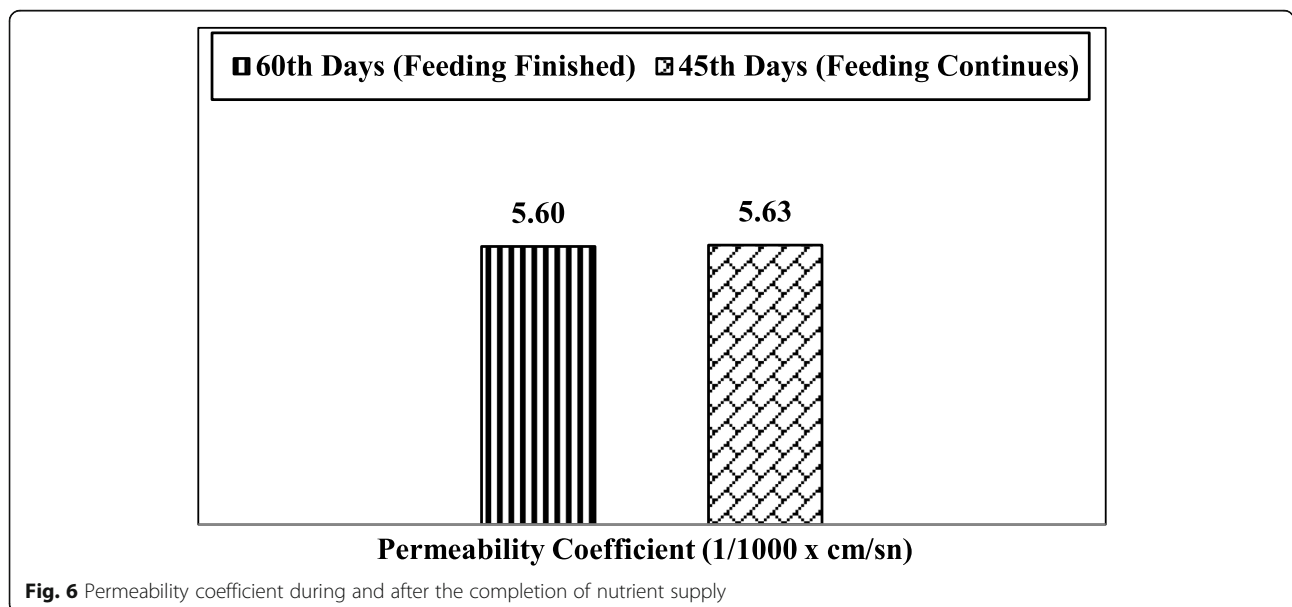


production of calcium carbonate by the bacteria are limited. On inoculating the soil with *Bacillus sp.* cementing occurs and this leads to an increase in cohesion and internal friction angles of the soil, this increase is recorded within the first 15 days, after which no significant increase in internal 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 by 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 (Fig. 4).

Harianto et al. (2013), Hariana et al. (2018) and Osinubi et al. (2019) reported the utilization of *Bacillus* leading to 80%, up to 13 times and up to 6.6 times

increase in soil-bearing capacity values respectively. While for this study, an increase of up to 1.12 times in internal friction angle and 25 times in cohesion value was observed after 45 days.

Soil permeability

Permeability coefficient of the soil inoculated with *Bacillus sp.* was observed to plummet by 30.36 times

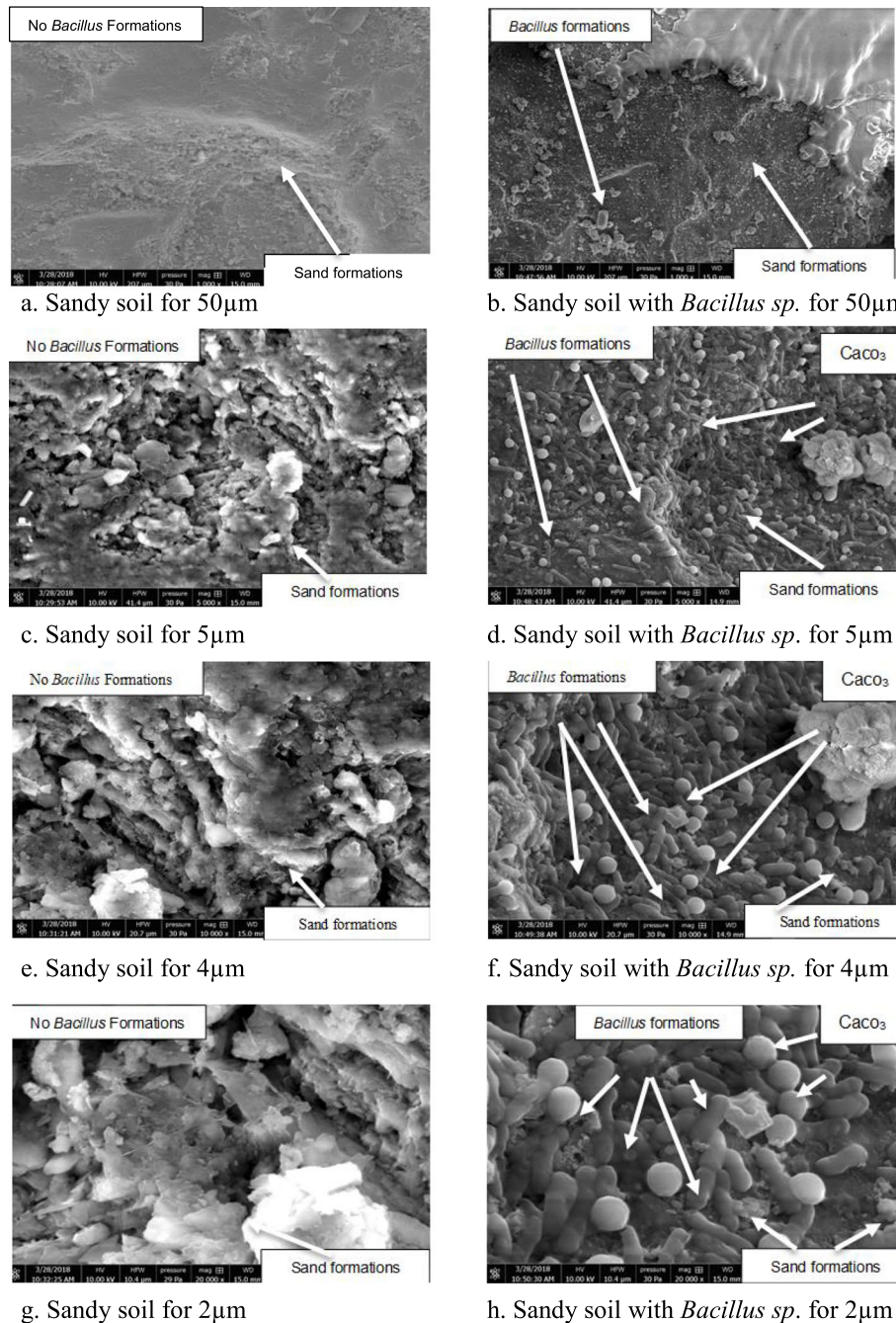


Fig. 7 The images of SEM before stabilization (a, c, e, g) and after stabilization (b, d, f, h) (Bagriacik et al., 2018)

after 45 days (Fig. 5), 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 (Fig. 6). Nemati and Voordouw (2003), Whiffin et al. (2007), Yasuhara et al. (2012), Harianto et al. (2013), Soon et al. (2014) and Umar et al. (2016) reported 98%, 22–75%, 60–70%, 80%, 90% and 90% improvements in permeability respectively while for the current research of ours we observed improvement in permeability value of up to 30.36 times after 45 days.

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 controlled soil calcium carbonate was not present (Fig. 7). The result of X-ray diffraction analysis (XRD) of both the treated and controlled soil is presented in Tables 1 and 2 and Fig. 8, it indicates that ratio of calcium carbonate increases considerably in the soil treated with *Bacillus sp.* and this leads to the alteration in the chemical structure of the soil.

Conclusion

We aimed in this study to devise an alternative technique of weak soil stabilization using *Bacillus sp.* Obtained results are presented below.

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

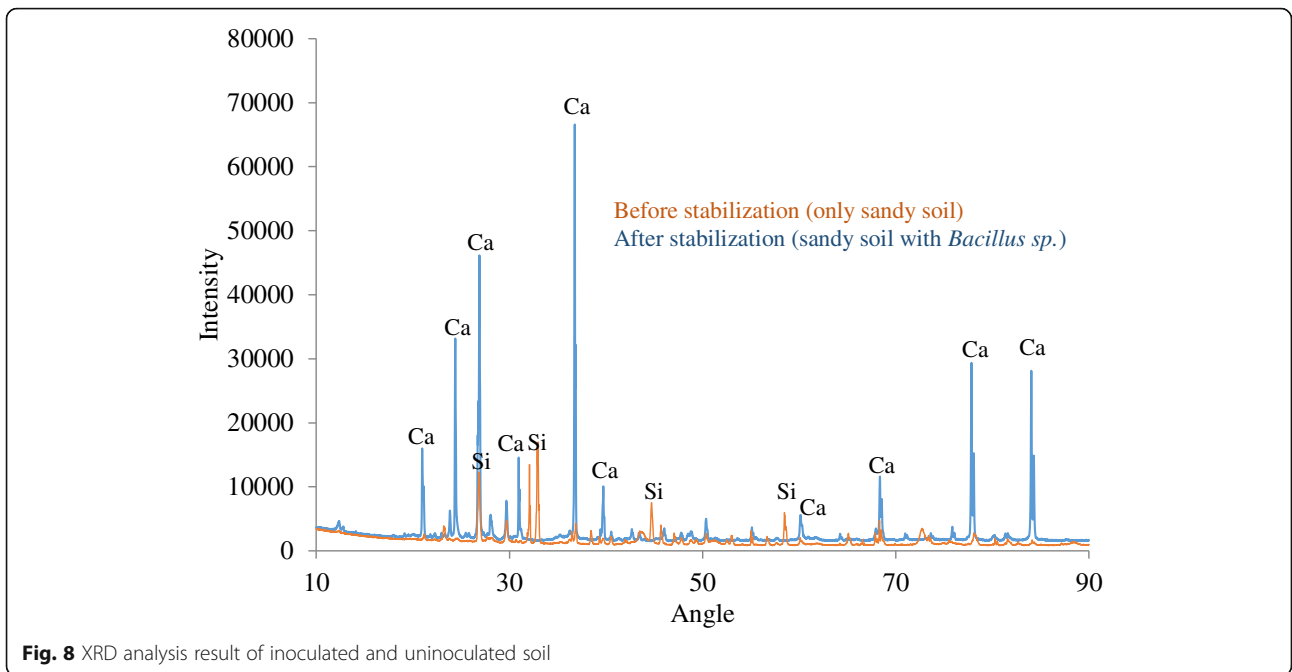
Component	Sandy soil (%)
SiO ₂	96.15
Al ₂ O ₃	2.10
Fe ₂ O	0.6
MgO	0.04
CaO	0.22
MnO	-
K ₂ O	0.39
Na ₂ O	0.07
TiO ₂	-
P ₂ O ₅	-

A short time after improvement with *Bacillus sp.*, no significant improvement was observed when the results were compared with the control samples. It showed that *Bacillus sp.* needed more time to produce calcium carbonate, and it was understood that such improvements would not yield fruitful results in a short time. However, there have been significant improvements in the engineering properties of the soil over time. This situation is thought to be due to the increase in the attraction force between the sand grains due to the calcium carbonate produced. It was observed that the intergranular attraction forces due to the produced calcium carbonate increased and the gaps decreased. Thus, the resistance of the soil to volume changes increased and the hydraulic permeability decreased. In addition, a more stable structure was created against settlements and the risk was reduced in the sand that has the potential to liquefy during an earthquake. There was no decrease in the improvements in the engineering properties of the soil after the nutrient that should be given to the bacteria to produce calcium carbonate was cut off. This showed that the improvement with *Bacillus sp.* was sustainable. Alternative methods are needed due to the fact that the traditional additives (such as cement, fly ash) used in the improvement of sandy soils emit CO₂ to nature during the production and use and their costs are high. With this study, it was determined that sandy soils can be improved with *Bacillus sp.*, an environmentally friendly and economical approach, and this improvement is sustainable.

Methods

Physical and chemical characterization of the soil

Sandy soil samples were collected from the riverbed of Seyhan river (37° 05' 37.3" N 35, 35° 12' 31.7" E) Adana, Turkey, and were oven-dried. Physico-chemical



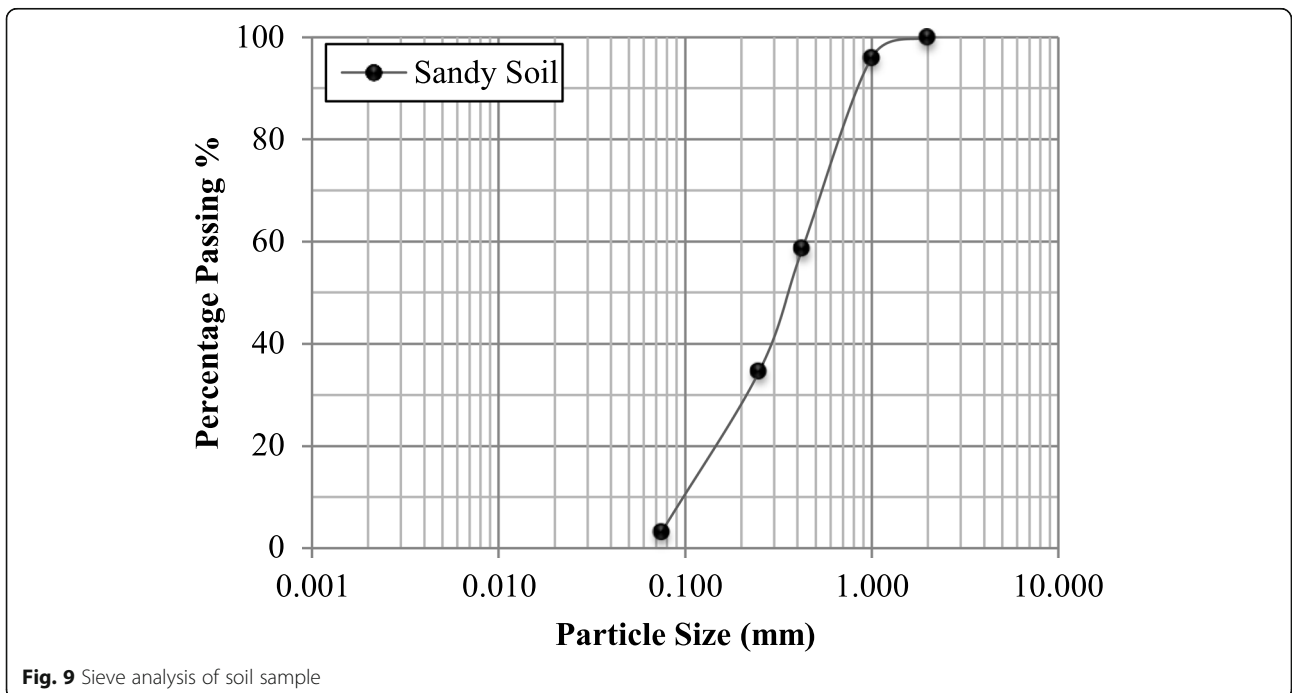
characterization of the collected samples was conducted according to [ASTM D 6913-04](#) established protocols.

The particle size distribution of the sample was found to be 53.6% fine grain and 46.4% medium grain sand (Fig. 9). The soil was found to have 26.80 kN/m³ specific gravity, 15.03 kN/m³ minimum dry specific gravity and 17.06 kN/m³ maximum dry specific gravity. Tables 1

and 2 presents the physical and chemical properties of the soil sample under study.

Isolation and characterization of microorganisms

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 g from each of the samples was homogenized in 10 ml serum physiologic buffer, the resultant suspension was incubated at 85 °C for 15 min with the aim of eliminating non-spore forming bacteria. One hundred microliters of the heat-treated sample suspension was spread plated on urea agar and incubated at 37 °C for 24 h.

To determine the urea hydrolysing ability of the isolates, the isolates were inoculated on a urea agar (gL⁻¹, 20 g urea, 9.5 g Na₂HPO₄, 9.1 g KH₂PO₄, 0.1 g yeast extract, 0.01 g phenol red, 12 g agar pH, 6.8) slant and incubated at 37 °C for 2–5 days. Development of pink colour was considered a positive result. Positive strains were stored in glycerol stock for use in subsequent experiments.

The identity of the isolated bacteria was determined through the amplification and sequencing of its 16s rRNA gene using 27f/519r primers. The 16sRNA gene sequence of the bacteria was aligned with known sequences using the BLAST algorithm in the NCBI database. The bacterium was identified as *Bacillus sp.* (accession number NR_114919.1) (Fig. 10).

Media preparation

The urea medium for the experimental study was prepared by dissolving 3 g nutrient broth powder, 20 g urea, 10 g NH₄Cl 10gL⁻¹, 2.12 g NaHCO₃ in 1 l of distilled water. The pH of the solution was adjusted to 6.0 and autoclaved at 120 °C for 15 min and 20 ml of calcium chloride solution (CaCl₂·2H₂O 18.5 g/100 mL) was added to the autoclaved medium to support CaCO₃ formation (Canakci et al. 2015).

Treatment of sandy soils

Urease-positive strain selected for use in the experiments was inoculated into the urea medium. The bacteria produced were precipitated by centrifugation at 9,000 rpm for 5 min while in the logarithmic phase and the pelleted part was taken and resuspended in a fresh urea medium. The final bacterial density was determined as 1.2 × 10⁶ cfu/ml by counting with the Petroff-Hausser slide. Twenty millilitres of the prepared bacterial culture was injected into the soil samples. Soil samples used as the control group were treated with bacteria-free urea medium. Twenty millilitres of medium was added to all samples every 6 h for 15 days by passing it through a sterile filter of 0.22-µm diameter.

Direct shear test

Direct shear test was conducted on the collected soil sample using the apparatus depicted in Fig. 11 to determine the shear properties, cohesion and internal friction angle (ϕ) 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 60 × 60 and a height of 50.8 mm. 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 D 3080 standards.

Bacillus sp. was inoculated into the soil sample. The inoculated soil was dried in an oven fed on the

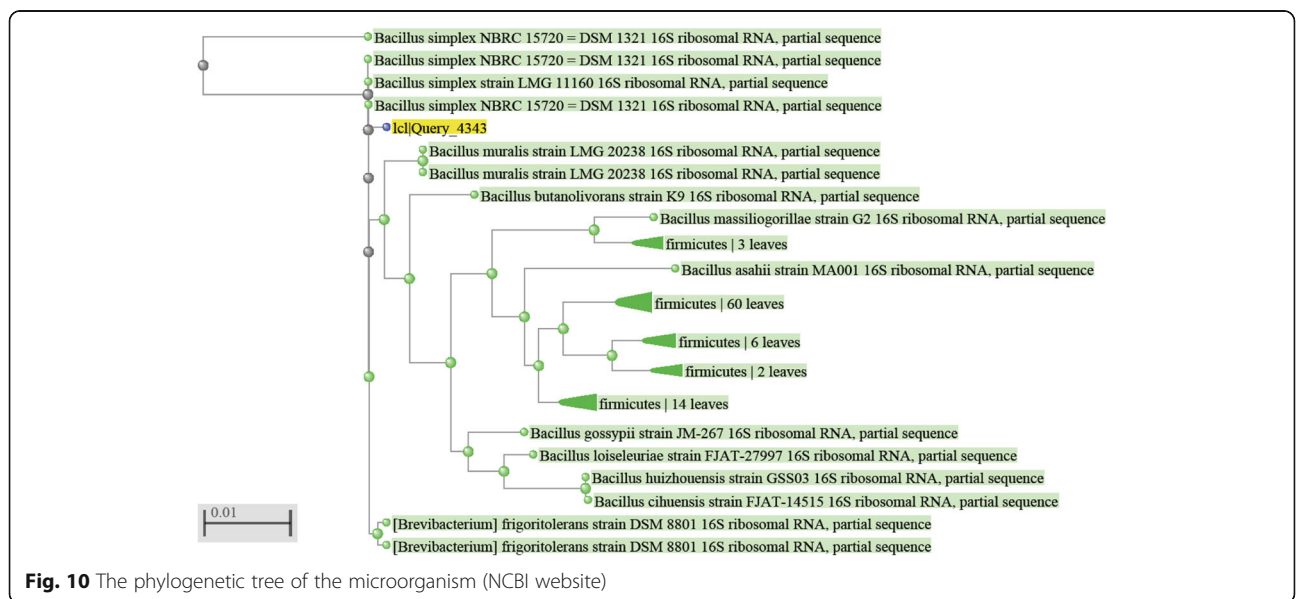


Fig. 10 The phylogenetic tree of the microorganism (NCBI website)



a. Preparation container

b. Test cup assembly

c. Soil sample introduction



d. Injection of Urea medium



e. Sample protection



f. *Bacillus sp.* growth medium



g. Vertical load application on sample



h. Direct shear test machine

Fig. 11 Direct shear test setup

1st, 2nd, 5th, 15th, 30th and 45th day of inoculation, cohesion (c) and internal friction angle (ϕ) of the soil were determined. Uninoculated soil sample served as a negative control.

Permeability test

The permeability of the soil sample under analysis was determined in accordance with [ASTM D2434-19](#) standard (Fig. 12). Readings were taken throughout the



Fig. 12 Permeability test setup

permeability test at different time intervals and permeability coefficients determined.

The microstructural analysis

In the study, SEM analyses were performed to observe the formation of *Bacillus sp.* on sandy soils. The SEM of each sample was performed by the FEI-QUANTAFEG-650. The XRD of each sample was obtained using a Rigaku Miniflex XRD equipped with radiation. The material phases were identified through PDXL software using the current database.

Acknowledgements

Not applicable.

Authors' contributions

BB: Conducting experiments, article writing, literature search and writing, evaluation of results and review of article language.
ZKS: Literature search and writing and article language review.
FMU: Isolation and characterization of *Bacillus sp.*
ESY: Isolation and characterization of *Bacillus sp.*
SD: Article writing, evaluation of results and article language review.
The authors read and approved the final manuscript.

Funding

The authors declare that funding was not received for this publication.

Availability of data and materials

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

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 18 March 2021 Accepted: 27 July 2021

Published online: 09 September 2021

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