
AUT Journal of Civil Engineering

AUT J. Civil Eng., 4(1) (2020) 17-26
DOI: 10.22060/ajce.2019.15662.5546

Biological stabilizers for different dispersive clayey soils

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ABSTRACT: Dispersive clays are a particular type of soil material in which the clay fraction erodes in the presence of water by a process of deflocculating and could cause significant problems in geotechnical and geo-environmental projects. Dispersive soils have been found to exist in various types of climates, especially arid climates, which the extent of dispersion mostly depends on mineralogy and clay chemistry. Bentonite, kaolin, and fibrous clay soils are among the most important and useful industrial materials. Therefore, clay dispersion potential was investigated by adding dispersive materials (Sodium hexamethaphosphate) and performing shear strength, crumb, double hydrometer, pinhole tests and chemical experiments. Stabilization practices using biological methods were done with Bacillus sphaericus and Bacillus pasteurii after soil divergence to assess the impact bacteria strains on soil improvement parameters. Bases on chemical properties, Sodium Adsorption Ratio and stabilization potential was in the order of kaolin>sepiolite>bentonite; the trend changed in accordance with clay CEC. The role of produced carbonate was prominent in improving mechanical and dispersivity properties. Soil chemical (exchanging Na ions) and mechanical (cohesion and friction angle) characteristics ameliorated by B. pasteurii further than B. sphaericus. In general, biological stabilization may replace conventional soil improvement methods as an eco-friendly and efficient way toward sustainable development.

Review History:

Received: 2019-01-18
Revised: 2019-04-03
Accepted: 2019-04-05
Available Online: 2019-04-05

Keywords:

Dispersion Soils
Clay Minerals
Chemical tests
Biological Stabilization
Bacteria

1. INTRODUCTION

Dispersive soils, as a problematic soil for different applications, are prevalent over wide areas of the world, especially in arid and semi-arid regions. Dispersive soils are comprised from clays particles contain high content of sodium ion in their adsorptive ions that can be easily washed by the waters with low salt contents [1] Generally, a saline soil becomes sodic through the leaching of salt (e.g. sodium chloride), usually over many thousands of years. As salt is washed down through the soil, it leaves some sodium behind bound to clay particles displacing other elements such as calcium. When there is excess sodium, soil swells and clay particles disperse when it contacts with water, rather than sticking together, causing the soil structure to slump and collapse [2 and 3] On the other hand, the presence of water will overcome and eliminate the inter-particle forces that the particles will move apart, forming a dispersed colloidal solution. The separated particles would move even with a slow water flow. However, non-dispersive soils are eroded only when the water flow is strong enough to overcome the inter-particle attractions. Tiny particles of dispersed clay then block soil pores and cracks [4].

For clay soils, clay surfaces typically carry a net negative charge, which is neutralized by a diffused ion cloud in which the concentration of cations increases and that of the anions decreases as they approach the surface.

This phenomenon is referred commonly as a diffuse double layer. This electrical layer consists of the surface charge and compensating counterions that form a surrounding ion swarm. The thickness of the diffuse double layer depends on the nature of exchangeable cations and electrolyte concentration of soil solution [5]

The behavior of two pure clays (Illite and bentonite) and two soil clays in aqueous suspension was investigated by [6]. As the iconicity index decreased in the following order $\mathrm{Li^{+}>Na+>K^{+}>Mg^{2+}>Ca^{2+}>Sr^{2+}>Ba^{2+}}$ the tendency to co-valency increased and, hence, the predisposition to break the clay-cation bonds in water decreased. Soils with smectitic mineralogy and high cation exchange capacity dispersed less than soils dominant in illitic and kaolinitic clays. A high correlation ($r^2=0.72$) was obtained between the dispersed clay content and Zeta potential of all soils with different treatments confirming that the net charge on the soil surface available for water interaction controls the dispersion-flocculation phenomena.

Bacillus pasteurii and *Bacillus sphaericus* are among the urease producing bacteria in the soils and urea is a nitrogen

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*Corresponding author's email: abbaslou@sirjantech.ac.ir source for them [7]; these bacteria are widespread in the soil environment, and perform carbonate precipitation which produced CO_2 is trapped in $CaCO_3$ preventing CO_2 emission into atmosphere (equations (1), (2), (3) and (4)) [8].

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \quad (1)$$

$$NH_2COOH + H_2O \rightarrow NH_3 + CO_2 + H_2O \quad (2)$$

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+ \quad (3)$$

$$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3 \quad (4)$$

All studies of soil stabilization by production of biological calcium carbonate has done on sand [e.g. 9 and 10] or silt and loam (e.g. 11 and 12]. Also, most studies have done to investigate stabilization impact on physical soil properties. Soil microorganisms play an important role in formation of fine grained soils and change the behavior of coarse-grained soils. Hence, despite their relevance in influencing the properties and behavior of soil, less work has been done to explore the importance, relevance, usefulness and application of these microorganisms in geotechnical engineering [13]. This is the first published study to achieve bio-improvement of clayey soils via the application of bacterial cultures. Therefore, the study assesses the dispersivity of different clay soils (kaolin, bentonite and fibrous) along with comparison of the biological stabilization with the conventional stabilization methods. In addition to, the effect of bacteria strains (*Bacillus sphaericus* and *Bacillus pasteurii*) on soil bio-amelioration will be investigated.

2. METHODS AND MATERIALS

2.1 Soil samples

To investigate the effect of clay types on the soil dispersion, three different clay soils were selected: 1) Kaolin soil from ore mine in eastern north of Iran, 2) Bentonite soils from eastern south of Iran, 3) Fibrous (sepiolite soil) clay mineral from eastern north of Iran.

2.2 Soil mineralogy analysis

To determine the composition of mineral deposits and to determine the mineral purity reservoirs, powder samples were prepared and studied by X-ray diffraction.

A Philips X- ray diffractometer (Model PW 1840-Copper target- scanning speed 0.5 degree per minutes- Voltage 4 kV- Amperage 20 mA) was used to record diffractograms between 2 to 80 degrees (2θ). Fig.s 1, 2 and 3 illustrate the main minerals of kaolin, bentonite and fibrous clayey soils, respectively.

2.3 Soil chemical properties analysis

Calcium carbonate equivalent (CCE) was determined for all the samples [14]. Cation exchange capacity (CEC) was measured in the sodium acetate at a pH of 8.2 [15]. SAR and ESP of the soil samples were measured using laboratory tests as described by [16].

2.4 Soil physical and engineering properties measurement

The engineering properties were determined according to ASTM standard methods. Atterberg limits (i.e., liquid limit, LL, and plastic limit, PL) were determined in accordance with the ASTM D-4318 Standard Test. In order to determine the compaction characteristics, the standard Proctor compaction test was performed according to ASTM D-698 on the clay soils. Particle size distribution of the samples was obtained by the hydrometer analysis (ASTM 2006, D-422). The specific gravity of soil solids (Gs) was determined according to ASTM D-854. To check the strength parameters of soil samples, the direct shear test was performed (ASTM D3080 - 04) as below:

$$\tau_{max} = c + \sigma_n \tan\phi \quad (5)$$

which is often referred to as the Mohr-Coulomb equation in classical soil mechanics where c = cohesion, ϕ= angle of internal friction, and σ and τ_{max} are the normal and maximum shearing stress, respectively, acting at a point on the failure surface. To estimate cohesion and friction angle for sol samples using shear strength, specimens were compacted under standard compaction by an optimum soil moisture content. The specific surface area (SSA) was determined using the BET (theoretical background of the adsorption isotherm equation of **Brunauer**, **Emmett** and **Teller**) method described by [17].

2.5 Dispersive sample preparation and experimental methods

The clay samples were mixed with 4% Sodium hexametaphosphate (Calgon) with optimum moisture content; samples were incubated 30 days and blended every day. The following tests were conducted to evaluate the dispersivity of soils:

2.1.1 Soil dispersion test/aggregate stability test/crumb test-Astm D6572

Overall, a very useful test to indicate if the soil is sodic and dispersive by immersing fragments in fresh water. Cloudy or muddy water is an indication that the soil is dispersive.

2.1.2 Pin Hole test-ASTM D 4647-93

In the pinhole test, distilled water is allowed to flow through a 1.0 mm diameter hole drilled through a compacted specimen. The water becomes muddy and the hole rapidly erodes in dispersive clays. For non-dispersive clays the water is clear and there is no erosion.

2.1.3 Double Hydrometer test-ASTM D4221-99

The particle size distribution is first determined using the standard hydrometer test in which the soil specimen is dispersed in distilled water with a chemical dispersant. A parallel hydrometer test is then made on a duplicate soil specimen, but without a chemical dispersant.

2.1.4 Chemical Tests

Soil sodicity represents the combined effects of (1) salinity as measured by electrical conductivity of the soil, and (2)

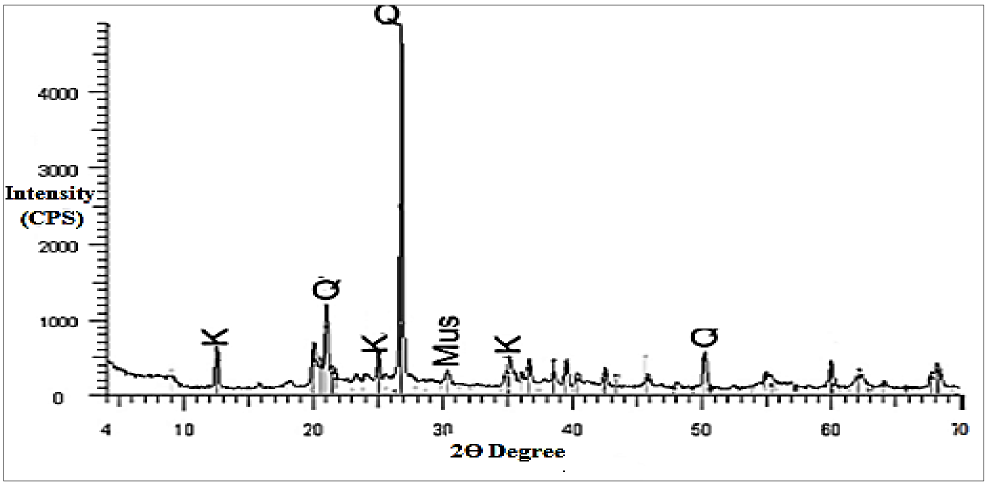

Fig. 1- X-ray diffraction of Kaolin soil containing kaolin (K), muscovite (Mus) and quartz (Q)

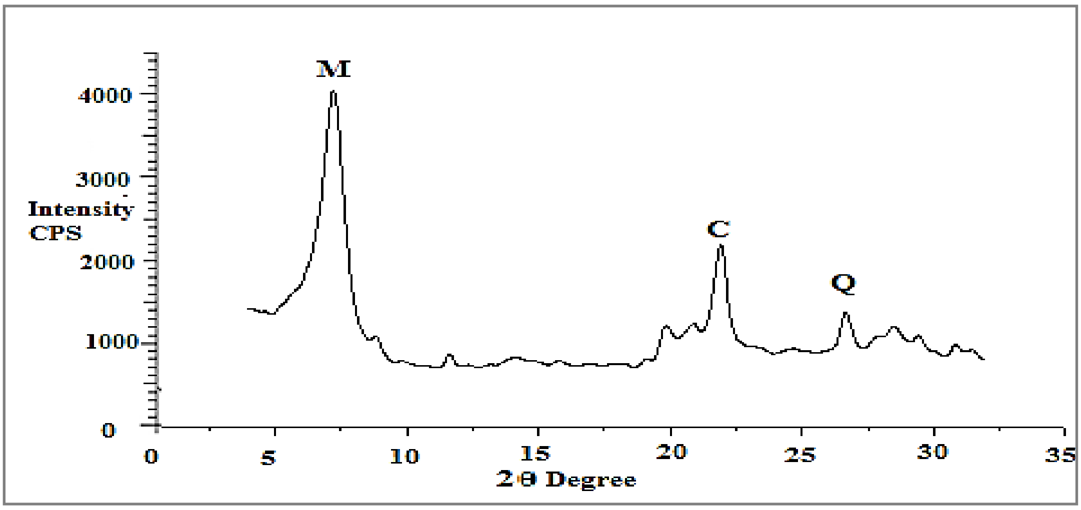

Fig. 2- X-ray diffraction of bentonite soil containing montmorilonite (M), cristobalite (C) and quartz (Q)

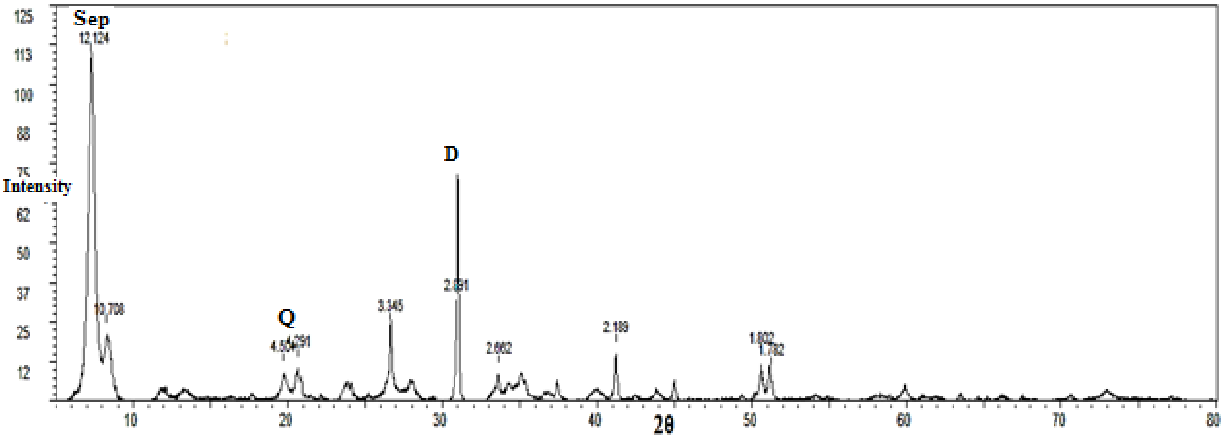

Fig. 3- X-ray diffraction of sepiolite (Sep.) soil with quartz (Q) and dolomite (D) impurities

soluble Na^+ concentration relative to soluble divalent cation concentration in soil solution, that is sodium adsorption ratio (SAR), or exchangeable sodium percentage (ESP). SAR is calculated by using Eq. (6):

$$SAR = \frac{CNa}{\sqrt{\left[\frac{CCa + CMg}{2}\right]}} \tag{6}$$

Where C represents concentrations in soil solution in terms of mmol$_c$ liter^{-1} (mmol$_c$ liter^{-1} = meq liter^{-1}) of the cations identified as subscripts.

ESP is calculated as the proportion of the cation exchange capacity occupied by the sodium ions. ESP is calculated from Eq. (7) by incorporating the values of exchangeable Na$^+$ and cation exchange capacity (CEC), both expressed as mmol$_c$ kg^{-1} or cmol$_c$ kg^{-1} (cmol$_c$ kg^{-1} = meq 100 g^{-1}) of the soil.

$$ESP = \frac{100\left(ENa\right)}{CEC} \tag{7}$$

ESP may also be calculated by replacing CEC in Eq. (7) with the sum of exchangeable cations such as calcium (ECa), magnesium (EMg), potassium (EK), exchangeable sodium (ENa), and aluminum (EAl), with all the cations expressed as mmol$_c$ kg^{-1} or cmol$_c$ kg^{-1} of the soil [18].

An ESP of 15 (~SAR 13) is generally taken to be the threshold below which the soils are classified as nonsodic, and above which the soils are dispersive and suffer serious physical problems when they subjected to water [6].

2.2 Soil stabilization and experimental methods

After dispersion process and ensuring dispersion, the soils biologically stabilized with *Bacillus sphaericus* and *Bacillus* pasteurii (Iranian Research Organization for Science and Technology, (PTCC) 1495 and (PTCC) 1645, respectively). Samples used for the experiments were heated in oven (T=150°C) to eliminate the indigenous bacteria. The cells were separately cultured into proper amount of sterilized medium containing the following l^{-1} of glass distilled water: nutrient broth (Bacto, 3g), ammonium chloride (10 g), sodium bicarbonate (2.12 g equivalent to 25.2 mM), supplemented with 1 % w/v filter sterilized Urea. Then, the inoculated mediums with bacteria were grown with agitation 190 rpm at 30℃ for 48-72 h and aeration (200 mL of media in a 1-L flask) till the optical density of 600 nm (OD$_{600}$) reached to desired ODs (1 and 2). Also, one flask containing the medium without the bacteria was prepared and incubated under the same conditions as a control sample for checking the contamination. CaCl$_2$ (0.01 M) was added to harvested bacteria solution as a precipitating agent before injection into the soil samples.

The clay samples were separately mixed with the different bacteria and ODs at optimum moisture content (pH 6.8 to 7) for a period of one month and the moisture checked other day by weighting. The homogenized soil mixtures were then used at different tests. For each test, the triplicate samples were prepared to verify the reproducibility of results. The average values of results employed in further computation and plotting of graphs. To analyze the flocculation of soils and soil stability, the following experiments were conducted to evaluate different effects of various types and concentrations of solutions on the dispersion of different clay soils as described in the upper sections:

- Shear strength-Pin Hole Test

-Chemical test

Table1- Chemical Characteristics of clay soils

Properties	Kaolin	Bentonite	Sepiolite
CEC cmol$_c$kg^{-1}	3	35	13
CCE %	15	25	20
SAR	1.5	15	4.3
ESP %	2.1	13.9	4

Table 2- Engineering and geo-environmental properties of clay soils

Characteristics	Quantity measured		
	Kaolin	Bentonite	Fibrous
Soil classification	CH	CH	CH
Liquid Limit %	60.37	131.95	164.57
Plastic Limit %	25.93	42.86	92.5
Plastic Index	34.44	89.09	72.07
Gs	2.63	2.6	2.35
SSA (m^2 g^{-1})	40	220	180
γ_{dmax} KN m^{-3}	17.3	17.5	16.3
ω_{opt} %	22	39	37.3

Morphologies of samples for detection of calcium carbonate composition were observed with a field emission scanning electron microscope (FESEM), MIRA 3-XMU model operated at 18KV and elemental analysis of sample was carried out using EDX (Energy Dispersive Analysis of X-Rays) model (Bruker X Flash 6130). Sepiolite soils inoculated with *Bacillus pasteurii* were scanned with Field Emission scanning electron (FESEM) equipped with an EDX device.

2.7 Statistical analysis

To identify significant difference among treatments, mean comparison were conducted based on Duncan Multiple Range Test (p<0.05). All statistical analyses were performed using SPSS software (Ver. 17.0) and graphs were drawn in Microsoft Excel 2010.

3. RESULTS AND DISCUSSION

3.1 Properties of clay soils

Clays are among the most widespread sedimentary rocks; due to their specific properties, clays are widely used in various industries and engineering applications. In all problems involving work in clay soils, the engineer has to accept their properties as they are and make the best of them by solving their problems. A major group of problematic soils in arid and semi-arid regions are dispersive clay soils.

Dispersive soils are clayey soils which are containing high percentage of exchangeable sodium ions and highly susceptible to erosion that considered as problematic soils for hydraulic structures application.

Table 3- Dispersion chemical experiments of clay soils

Soil Type	Exchangeable Na*	Exchangeable Na≠	Soluble Na*	Soluble Na≠	SAR*	SAR≠	ESP*	ESP≠
	Meq/100g							
Kaolin	3	100	1.1	75	1	21	1	33
Fibrous	41.8	299.7	41	115	2.3	33.9	3.25	23.1
Bentonite	503.8	616.5	448	660	12	40.8	14.4	17.6

***Before treatment by dispersing agent, ≠ after treatment by dispersing agent**

Table 4- the result of double hydrometer of dispersive clay soils

Soil Type	percent of finer 0.005 mm with dispersing agent (A)	The percent of finer 0.005 mm without dispersing agent (B)	Dispertion ratio (B/A) %	Classification
Sepiolite	0.0048	0.0025	52.08	Dispersive Soil
Kaolin	0.0048	0.0023	47.92	Dispersive Soil
Bentonite	0.0044	0.0023	50	Dispersive Soil

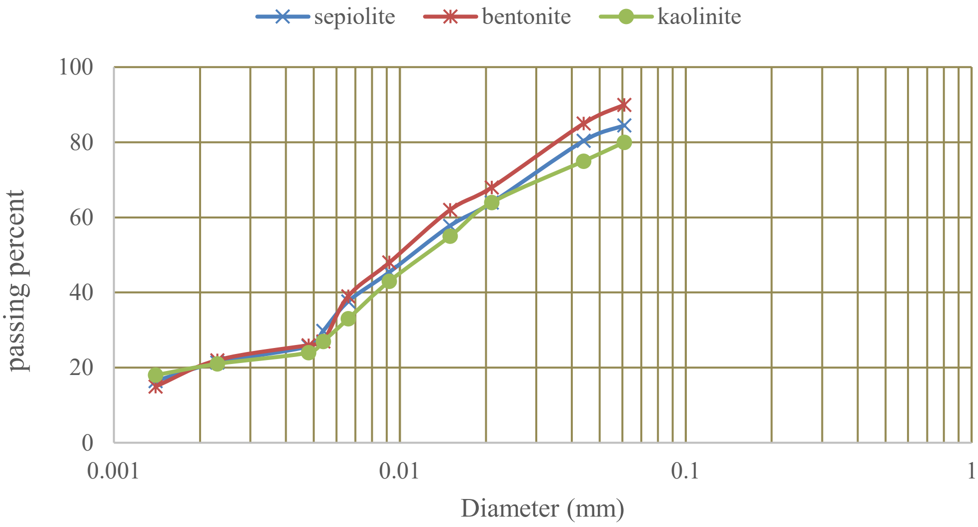

Fig. 4- Double hydrometer gradation curve of dispersive clay soils

The main chemical, Physical and engineering properties of studied clay minerals are presented in Table 1 and 2.

Kaolin clay soil has the lowest liquid limit, cation exchangeable capacity and specific surface area. Fibrous clay soil properties are the intermediate of the other two clay soils.

3.2 Properties of dispersive soils

The result of chemical experiments (Table 3) is presenting that soils have dispersed by adding 4% Sodium hexametaphosphate.

Concentration of Na ion in soil pore water layer is one of the important effective factors on soil dispersivity, because of sodium ion which is less electronegative due to its mono-valence. Potassium cations are also monovalent, but enter in the interlayer spaces of clay minerals and trapped due to their smaller sizes than Na ions. Presence of Na ions in soils lead to increasing osmosis potential and reducing attractive Van der Waals forces between soil particles. Usually soils which soluble salts concentration in pore water is less than 1 meq/lit (10 meq/100g) are not dispersive [19].

The result of Crumb test showed that sepiolite reacted more than other two clay soils in distilled water, while bentonite soil possessed the minimum dispersive reaction.

The results of Double hydrometer test are presented in table 4. Based on the result of the test (Fig. 4), the dispersivity is in the order of Sepiolite> Bentonite> Kaolin; However the differences were not remarkable among clay soils.

The obtained results of Pin-Hole test according to standard criterion are shown in Table 5. Besides the chemical tests, the Pin-Hole test is one of the most reliable methods for assessing soil dispersion. According to Pin-Hole test, soils were dispersed as an order of sepiolite~kaolin> Bentonite.

The results of different tests conducted on all the clay soil samples indicated different outcome on dispersivity criteria.

Table 5- The Pin-Hole results of clay soils after dispersion

Soil Type	Water head (mm)	Time (min)	Final flow rate (ml/s)	Hole diameter (mm)	Classification
Sepiolite	50	5	2.07	3	D-1
Kaolin	50	5	2.48	2.8	D-1
Bentonite	50	10	2.18	>1.5	D-2

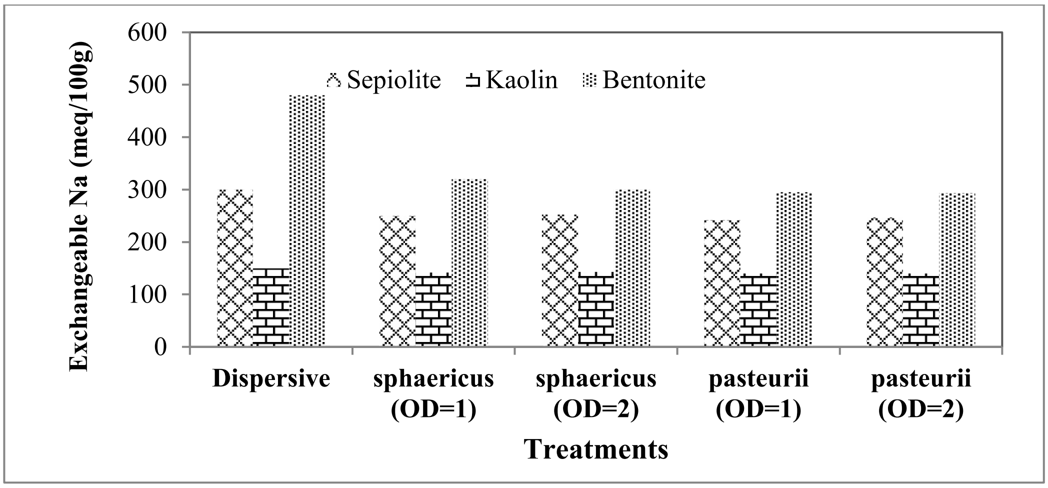

Fig. 5- Variation of Exchangeable Na with different biological stabilizers after dispersion

It is necessary that the dispersivity of soils be investigated by different methods. In general, the potential of dispersivity of soils could be as Kaolin> Sepilite>Bentonite soils. It can be concluded that bentonite soils with high CEC may adsorb high Na concentrations and endure earlier dispersion.

Rasheed [20] studied the relation between soil properties and dispersion ratio. Correlation coefficient between dispersion ratio (DR) with sand, silt and bulk density was positive, value was 0.4979, 0.0126 and 0.7536, respectively, while with clay and specific surface area (SSA) the correlation coefficient value were −0.7281 and −0.4466, respectively.

The study of the influence of mineralogy and pore fluid composition on the slaking rates of three silty clays containing 20 percent kaolin (Hydrite R), illite and montmorillonite at low and high SAR values, but at constant concentration of pore fluid (0.01 N), showed that slaking rates are higher for the low plasticity and highly flocculated soil [21]. The soils with high concentrations of Na^+ ions relative to Mg^{2+} and Ca^{2+} ions which are generally dispersed and possessing low permeability values showed a lower degree of slaking rate. It can be concluded from these observations that clay type and clay amount, pore fluid composition, water content, density and soil structure control the slaking rates of soils.

Free swell tests carried out on a large number of natural and artificial soils [22-26] showed that for highly dispersive soils the value of constant water uptake (osmotic swell) is a function of the type and amount of clay mineral.

3.3 Biological Stabilization of dispersive clay soils

Biological stabilization was done by *Bacillus pasteurii* and *Bacillus sphaericus* with the aim of assessing and comparing the impact of different urease bacteria strains on soil stabilization. The trend of biological stabilizers on soil flocculation by Pin-Hole test showed an order of *B. pasteurii* > *B. sphaericus*. There were not observed a significant trend between optical densities (OD=1 and OD=2) of bacteria strains.

Variations of exchangeable and soluble sodium after treatment with biological stabilizers for different dispersive soils are drawn in Fig. 5 and 6, respectively. By biological stabilization of soils, sodium exchanged by Ca of biologically produced carbonates; it means that high amounts of Na exchanged by Ca which the values of exchangeable Na have decreased. Based on amount of sodium exchanged on clay sites, the soluble Na was increased in the order of *B. pasteurii* > *B. sphaericus*. As it has shown in Fig.s 6 and 7, the OD have non substantial impact on exchangeable Na and soil flocculation.

Following a concentration increase in bivalence ions of calcium around clay minerals, these ion replaced sodium ion which is less electronegative due to its mono-valence, thus, thickness of double layer decreases and leads to an increase of attractive forces between minerals.

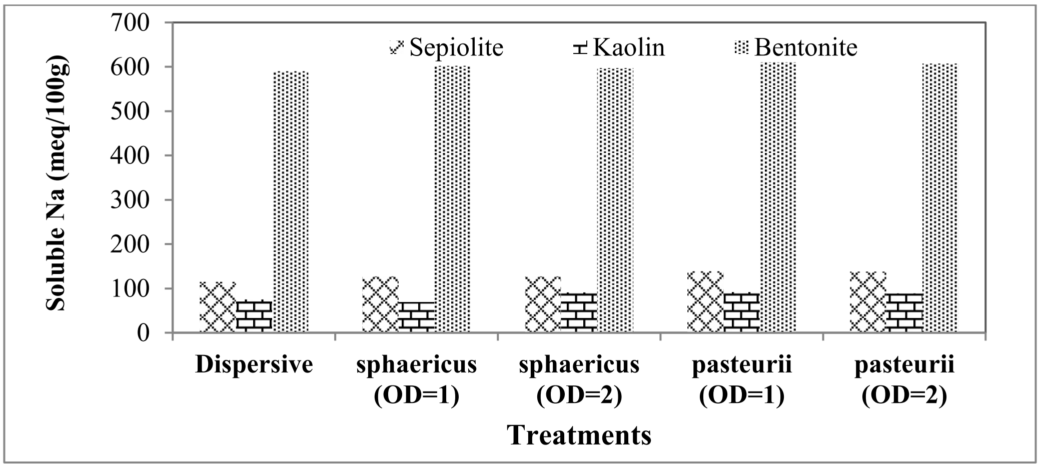

Fig. 6- Variation of soluble Na with different biological stabilizers after dispersion

Microbially calcite precipitation (MCP) describes the formation of calcium carbonate minerals from a solution due to the presence of microbial cells, biosynthetic products, or metabolic activity [27]. To test the idea of effect of MCP bacteria strains on soil strength, the shear strength parameters (cohesion and friction angle) of different clay soils were measured before dispersion, after dispersion and also after stabilization by bacteriastrains (Fig. 7).

In general, there are no significant relations between dispersive and non-dispersive soils in all clay soils regarding soil strength parameters (cohesion and friction angle), while after biological stabilization; the difference was significant compared to dispersive and non-dispersive soils for both bacteria strains and also ODs. Terzis et al. [28] studied the effect of treatment conditions on the fabric characteristics of bio-improved sand for various treatment patterns and addressed the mechanical response for three typical cases of samples obtained during laboratory experimentation with MCP. They revealed improved mechanical properties were obtained in terms of angle of internal friction and cohesion. Van Paassen [29] reported values of increasing unconfined compressive strength (UCS) and peak shear strength for drained and un-drained conditions when the calcite content increased, establishing a trend between the precipitated mass and the expected range of shear resistance.

Among biological treatments, *Bacillus pasteurii* (OD=2), *Bacillus pasteurii* (OD=1), *Bacillus pasteurii* (OD=1) had more influence on soil improvement strength parameters for sepiolite, kaolin and bentonite clay soils, respectively. As a consequence, the role of *Bacillus pasteurii* on soil strength parameters was outstanding rather than *Bacillus sphaericus*. However, there was no remarkable trend between raising cell number or growth of the culture (Optical density: OD) and bacteria effects; it means the bacteria may fulfill their role in an identified initial amount and no need to increase the number of bacteria cells before mixing with the soil sample.

Usually, the strains of Bacillus pasteurii, are commonly used for bio-cementation [9, 30, 31, 32, 33 and 34]. - These Bacteria belong to Risk group 1 with low individual and community risk.

The bacterium *Bacillus sphaericus* has been used for stabilizing dispersive loamy soils by [12]. The dispersivity of the treated samples decreased as the curing time and bacterial cell density of the treated samples increased.

Sarmast et al. [34] used bacterial species (*Sporosarcina pasteurii* and *Sporosarcina ureae*), for cementation of sand grains and infilling of pore spaces by CaCO3. Micro-morphological observations showed a high degree of calcite crystals' bridging, coating on sand particles and as well infilling of pore spaces. *S. pasteurii* has been thus recommended for being used in stabilization of sand dunes; due to its significant effects on CaCO3 deposition and as well on sand grain cementation.

In a general conclusion, biological stabilization treatments due to (1) low contamination rather than chemical stabilizers in their production, (2) their eco-friendly reactions in soil environment and no change in ecosystem, and (3) the appropriate performance in soil improvement characteristics and comparable to chemical and other soil treatments, could be used in dispersion reduction. On the other hand, they will be proposed as a reliable and efficient choice for soil amelioration in regard with engineering, chemical and physical properties to reach sustainable development objectives.

To demonstrate the carbonate amount changes, the CCE was measured for different clay soils and also after treatment by bacteria (Fig. 8). The *Bacillus pasteurii* treatments improved soil strength parameters (Fig. 7); on the other hand, *B. pasteurii* increased the MCP more than *B. sphaericus* (Fig. 8). However, the raising calcium carbonate amounts among clay soils types were significant, but between bacteria were not remarkable.

Fig. 9 shows the SEM image analysis of sepiolite soil specimen (Fig. 9a) and sepiolite soil inoculated with *Bacillus pasteurii*. Fibers of sepiolite were detected clearly in Fig. 9a. As shown in Fig. 9b, in which the tiny amorphous calcium carbonates accumulations were formed on the surface of

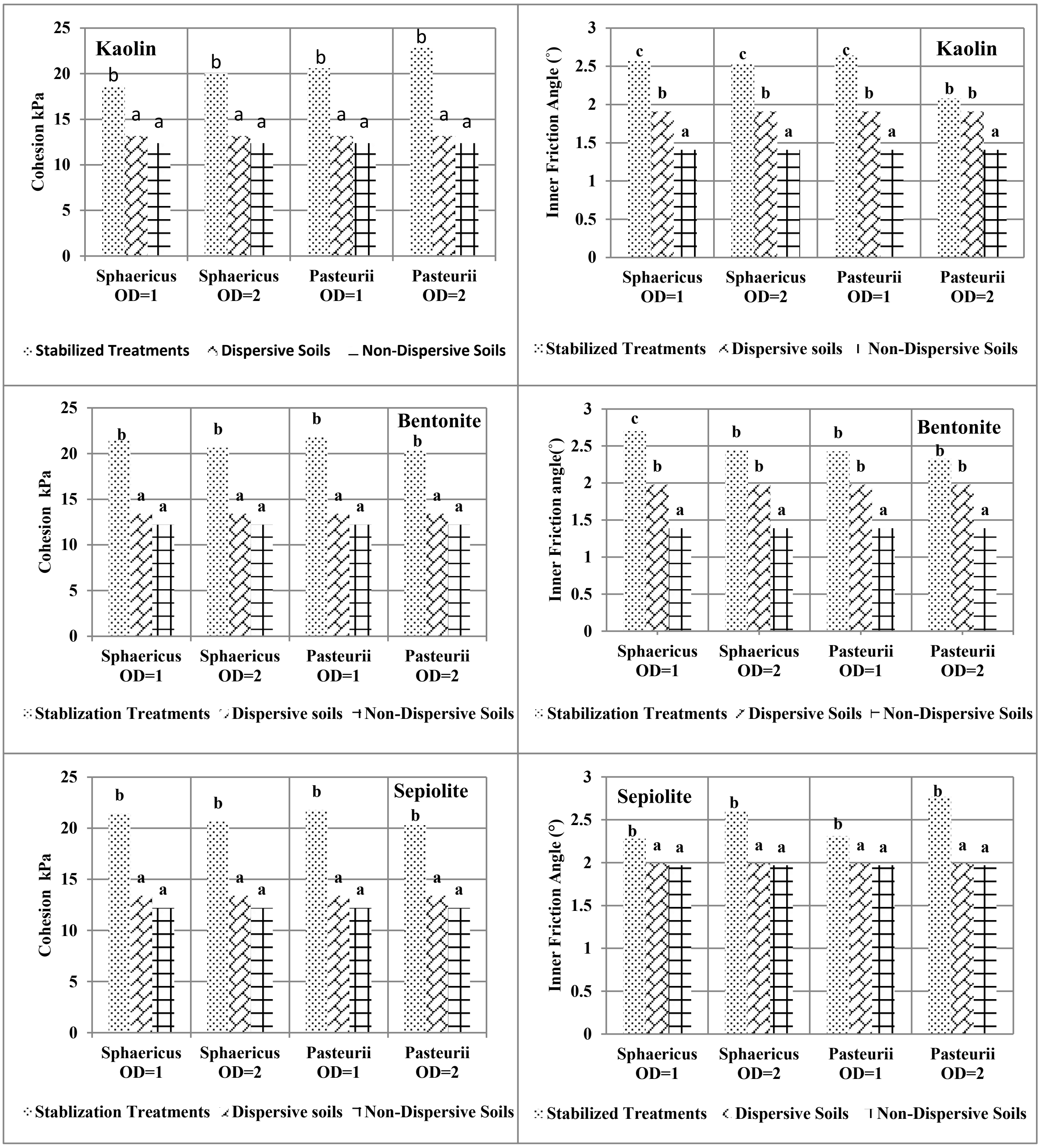

Fig. 7- Variation of shear strength parameters of different clay soils (Dispersive, Non-dispersive, and chemically stabilized (different letters (a, b, and c) indicate *significant difference* at *p* <0.05 (*Duncan's test*).

fibers. SEM-EDX analysis characterized the Si, Al, O, Mg, Fe (main elements of sepiolite clay mineral), and Ca (part of calcite mineral) as main elemental analysis.

4. CONCLUSION

Dispersion (the separation of soil into single particles) is governed by soil texture, clay type, clay chemistry, soil organic matter, electrical conductivity, sodium adsorption ratio, and exchangeable cations.

The capability of studied clay soils dispersion could be as Kaolin> Sepilite>Bentonite soils; so bentonite soils with high CEC may adsorb high Na concentrations and endure earlier dispersion.

The role of *Bacillus pasteurii* on improvement of soil strength parameters, chemical soil characteristics including exchangeable and soluble Ca and Na, and CCE percentage was outstanding rather than *Bacillus sphaericus* species. However, there was no remarkable trend between raising cell number

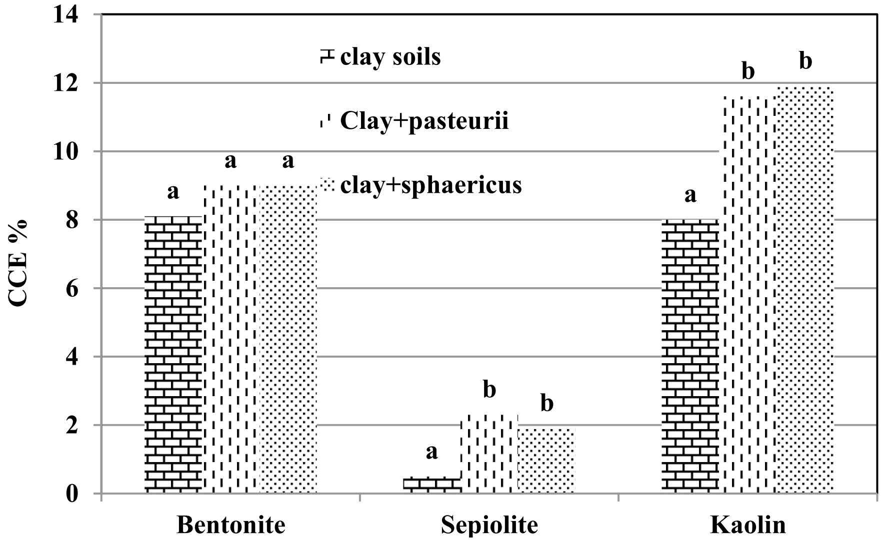

Fig. 8- Amount of CCE percentage for dispersive and biologically stabilized soils by *B. pasteurii* and *B. sphaericus* (different letters (a, b, and c) indicate *significant difference* at *p <0.05* (*Duncan's test*).

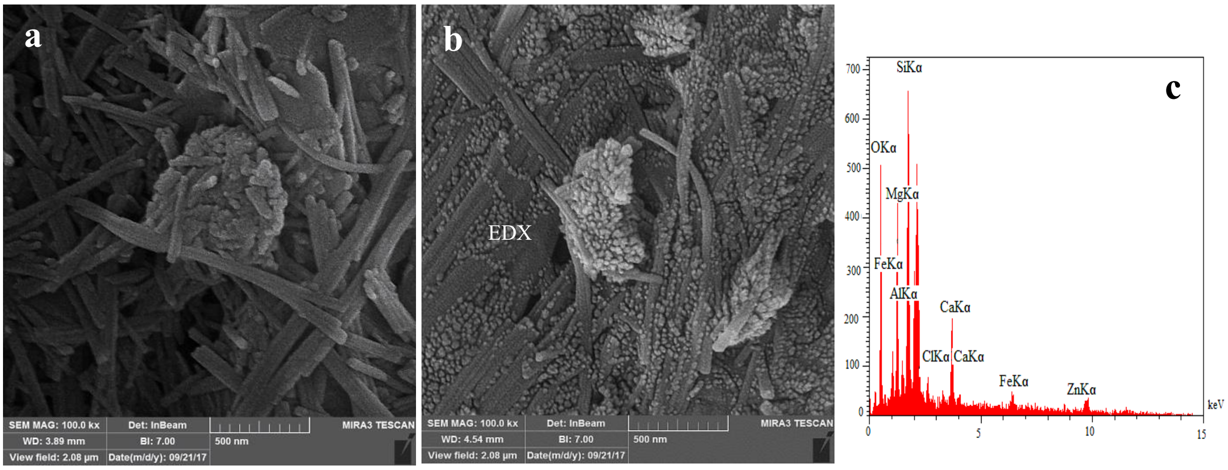

Fig. 9. SEM imaging of the sepiolite soil and soil inoculated with *Bacillus pasteurii* species: (a) fibers of speiolite soil, (b) particles of $CaCO_3$ detachment growing on sepiolite fibers; (c) SEM-EDX analysis of inoculated sepiolite soil with bacteria

or growth of the culture (Optical density: OD) and bacteria inflluences; it means the bacteria may fulfill their role in an identified initial amount and no need to increase the number of bacteria cells before mixing with the soil specimen.

Distinctive trends between dispersive and stabilized soils in regard with cohesion and friction angle values were obtained as far as the evolution of strength was concerned with respect to calcite content. Soil Strength enhanced by using MCP process mostly induced by Bacillus pasteurii species which could fulfill its role in an initial values of bacteria cell number. Soil strength parameters (cohesion and friction angle) increased after biologically stabilization due to calcite precipitation and soil flocculation.

Biological stabilization treatments due to appropriate performance, their ecofriendly reactions, and low contamination will be proposed as an efficient choice for soil improvement to reach sustainable development objectives.

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HOW TO CITE THIS ARTICLE

H. Abbaslou, H. Hadifard, A. Ghanizadeh, S. Kharazmi, Biological stabilizers for different dispersive clayey soils, AUT J. Civil Eng., 4(1) (2020) 17-26.

DOI: 10.22060/ajce.2019.15662.5546