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Improvement of Strength and Permeability Attributes of Silty Sand Type of Soils using Enzyme Induced Calcite Precipitation

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ABSTRACT. *Enzyme Induced Calcite Precipitation (EICP) is one of the soil stabilization techniques based on microbiological activity. In this technique, urea decomposes with the aid of urease enzymes in the presence of calcium chloride and produces calcite, which acts as a bio-clogging and bio-cementing material. In this study, test tube experiments are performed to assess the optimal amount of calcium chloride, urea, and urease enzymes to be used for engineering applications. The silty sand type of soil is treated with different proportions namely; P1 (0.1g urease enzymes, 0.375g urea, 0.9g* $CaCl_2$*), P2 (0.2g urease enzymes, 0.75g urea, 1.8g* $CaCl_2$*), and P3 (0.4g urease enzymes, 1.5g urea, 3.6g* $CaCl_2$*), to analyze their effect on strength and permeability attributes of soils after 14 days of curing time. It was observed that an increase in EICP content causes a substantial increase in shear strength particularly cohesion due to the bio-clogging phenomenon induced in soil particles by EICP which eventually leads to a decrease in permeability and inhibits the activity of urease. Overall, P3 yields higher cohesion (48 kPa) than P1 (40 kPa), P2 (43kPa), and untreated soil samples (31 kPa). Furthermore, P3 causes a significant decrease in permeability as compared to P2, P1, and untreated soil samples tested after 14 days of the curing period. The findings of the study suggest the successful implementation of EICP for soil stabilization.*

Keywords: Enzyme Induced Calcite Precipitation (EICP), permeability, shear strength, bio-clogging, bio-cementation

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1. INTRODUCTION

Soil stabilization using microbiological activity techniques progressed over the numerous past decades after the introduction of the concept of using calcite precipitation by live, urease-active bacteria, in 1991 by Ferris and Stehmeier. The researches show that microbial-induced calcite precipitation (MICP) is the most successful sustainable technique for refining the mechanical properties of poor soil, also known as bio stabilization or bio calcification (1–9). In MICP, hydrolysis of urea is catalyzed by uratolytic microbes i.e., bacteria it also helps in the formation of carbonate and in controlling pH of soil. Therefore, urease enzyme activity is most widely used in Calcium Carbonate precipitation or crystallization in the existence of Ca2+. The experimental results of different researchers show that the precipitated calcium carbonate ($CaCO_3$) strengthens the bond between sand particles and results in an increase in the strength and stiffness of sand (7–11). Even though microbial induced calcite precipitation is a widely used method for bio stabilization of soil, it has some drawbacks. To overcome the drawbacks of MICP an easy application and management of urease enzymes make Enzymes Induced Calcite Precipitation (EICP) technique more effective than microbial-induced calcite precipitation (MICP) which requires complex methods of applications, a specific environment for cultivation, and difficult management of bacteria (15–19). Enzyme Induced Calcite Precipitation (EICP) is one of the soil stabilization techniques based on microbiological activity. In this technique, urea decomposes with the aid of urease enzymes in the presence of calcium chloride and produces calcite, which acts as a bio-clogging and bio-cementing material (20–27).

Silty Sands usually require improvements in their geotechnical properties like shear strength and permeability, to make them suitable for construction work (28,29) Apart from shear strength, seepage is one of the major problems, as it not only affects the aesthetics but also affects the serviceability of the project. This study covers the

EICP process and represents yet another iterative refinement of the overall concept of bio-inspired soil stabilization, to improve the mechanical and cementing of the problematic soils, specifically Silty Sands (30–34) Also, the problem of seepage is observed with changing proportions of urea, calcium chloride, and urease enzymes in sandy soil. The chemical reactions of the additives promote the precipitation of calcium carbonate in a pour's medium. Calcium carbonate fills the void of soil and strengthens the bond between sand particles. This leads to a decrease in the permeability of the soil and increases its strength of soil (35–43). In short, the EICP technique helps in enhancing various mechanical properties of soil. Therefore, there is a need to examine the changes in soil permeability and shear strength against various proportions of urea, calcium chloride, and urease enzymes.

2. LITERATURE REVIEW

Numerous studies were already performed to improve different soil properties like hardness, strength, porosity, and permeability of different soils using EICP. The hardness, strength, compressibility, and permeability adjustments of treated soil are based on numerous natural and distinct characteristics of soil that track the enzymatic response with the essential chemicals to trigger calcite precipitation. Furthermore, the enhancement of different properties of the soil is continuously defined by certain actual soil properties. Minh & Cheng (44) has stated that the distribution of particle size, mineralogy, form, concentration, and the surface of aggregate particles affects the cementation process in the process of bio-mediated application. Positive results shown by this procedure in fixing leakage in the water holding structures, like dams and canals, and dropping the permeability of various soils by biologging (7,12). The procedure of using microbes to enhance the geotechnical properties of coarse soil began in Australia in 2001. As described by Starke et al., (45), engineers widely acknowledged the method after a sack of sand was converted into calcareous sandstone columns when handled by Australian research gathering. By the formation of particle binding materials by using microbial methods, bio cementation can be defined as the measure of soil improvement. It is often used to reinforce, stop, and improve soils in geotechnical design applications.

The application of urease enzyme derived from plants, in the fields of environmental and geotechnical engineering, was seen by Nemati & Voordouw, 2003, Neupane et al., 2013, (46) and Dilrukshi et al., 2016 (47). Recent research showed the feasibility of MICP to increase shear strength and decrease the permeability of tropical remaining soil and sand (48). The outcomes demonstrated a magnificent enhancement in shear strength of 96% at 0.5 M convergence of the binding reagents though, the enhancement in soil properties was hindered by the increasing amount of the chemical for example 1 M because of increasing saltiness which led to inhibitory impacts on the activities of microbes. The findings of Soon (2013) (48) are in concurrence with the findings of De Muynck (2010) (49) who demonstrated that higher convergence of binding reagents ordinarily expands the saltiness of the medium consequently hindering the movement of bacteria because of inhibitory impacts, however, the action of some certain microorganisms isn't generally influenced by the high saltiness of environment (12).

Whiffin (2007) reported a decrease in porousness from 22–75% of the underlying penetrability of the treated soil. A few specialists have used EICP for soil improvement purposes. Yasuhara (2012) (15) have worked on unconfined compressive strength and hydraulic conductivity of "Toyoura sand" treated utilizing different arrangements with different equimolar concentration of urea and calcium chloride. Urease catalyst enzymes with an announced activity of 2950 U/g was blended in different sand sample preceding presenting 0.50 M and 1.00 M urea 4 calcium chloride arrangements. These examiners reported strength improvement of 0.75 MPa and 1.6 MPa at an accelerated carbonate substance of 6% and 5%, separately, after 4 patterns of treatment with one pore volume of the 0.5 M arrangement in examples with 1 g of urease for each 300 g of soil.

Kavazanjian & Hamdan (2015) (50) did great work in the field of bio-geotechnical engineering. Their study shows an improvement in peak unconfined, compressive strength of around 0.5 MPa for a column of Ottawa 20/30 sand treated utilizing a blend and-reduced strategy to a carbonate substance of about 2.8 % with around one pore volume of an answer made from 1.4 M urea, 1.6 M calcium chloride, and 0.4 g/l of high action urease enzymes. Neupane (2015) (51) studied the allocation of carbonate precipitation at two different temperatures (5°C and 23.5°C) along 1-m columns of coarse sand that were confined to one treatment cycle through percolation of an EICP solution. These investigators observed an almost uniform distribution of carbonate precipitation (about 2 percent on average) at a lower temperature (5 °C) along the column of coarse sand. Nevertheless, along the depth of the column of sand treated at 23.5 °C, the carbonate content decreased from about 5 percent to zero. In these experiments, 1.00 M urea, 1.00 M calcium chloride and 15 g/l urease enzymes were used. By blending and progressive methodology. Oliveira (2016) treated sand, silty soil, and organic soil using EICP. The EICP arrangement they used, was made of 0.25M urea, 0.25 M calcium chloride, and 4 KU/L urease catalysts (relating to around 0.12 g/l of high-activity enzymes) in an underlying series of experiments. Additional experiments using 0.5 M urea and calcium chloride and 8 KU/L urease enzyme (relating to 0.23 g/l high activity enzymes) were carried out along these lines. They found that EICP treatment builds the strength of sandy and silty soil while it causes an impeding effect in the natural soil. A few specialists have considered impact of additives in the EICP arrangement on treatment viability. Magnesium chloride was added to the EICP technique by Lu (2016) (52). The urea classification was fixed at 0.50 M and the urease content was 1 g/l. The calcium chloride grouping varied from 0.25 M to 0.50 M and the convergence of magnesium chloride was altered accordingly, with the resultant target that the centralization of these two chlorides was equally to 0.50 M. They also reported that the development of aragonite along with more modest calcite precious stones advanced in the test tube experiment in presence of magnesium chloride, it also caused a higher precipitation proportion concentration of magnesium chloride of 0.20 M. Zhao (2015) (53) used poly-acrylic acid (PAA). After a single cycle of experimentation, these investigators registered an unconfined compressive intensity of about 5 MPa. The inspectors have confirmed that the addition of PAA to the soil resulted in 96 percent of the ammonium chloride by-product of the treatment phase being immobilized. Magnesium sulfate was applied to the EICP solution by Lu (2017) (52), setting the urea concentration at 0.50 M and varying the urease concentration from 1 g/l to 5 g/l. They observed that low magnesium sulfate concentration advanced aragonite production in test tube experiments, that the assisted mass increased with an increasing magnesium sulphate focus, and that crucial gypsum calculation was formed at a magnesium sulphate grouping of 0.10 M. They also found an increase with an increasing precipitation mass in unconfined compressive strength, up to an average of about 0.6 MPa in a silica sand treated with a 0.10 M magnesium arrangement. To remove the ammonium side effect. Putra (2017) also added the characteristic zeolite to the EICP response. For both urea and calcium chloride, they used EICP structure fixations between 0.5 M and 1.00 M and 1.00 g/l and 2.00 g/l fast movement chemical urease. They detailed that 10 g/l zeolite usage gives about 75 percent efficiencies of ammonium chloride expulsion depending on reagent focuses.

3. RESEARCH GAP

Numerous scientists have explored controlling the leakage and increment of the shear strength of soil with various strategies, particularly on bio geotechnical side MICP is generally used to improve the soil, yet MICP has some limitations just as impediments and disadvantages as referenced previously (45,54). Also, the EICP has some limitations like lack of nucleation sites (segment of $CaCO_3$ is precipitated in the pores), which may persist unproductive in the binding particles of soil. Likewise, numerous scientists have utilized EICP to research its impact on the conduct of various properties of soil, for example, compressive strength, bearing limit and development and withdrawal, and on criminal residue. Be that as it may, the utilization of EICP for drainage control and shear strength is a ground-breaking thought.

4. METHODOLOGY

The methodology of the experimental work started with the collection of samples and different tests which were performed for the completion of the project. The tests compose of two parts, in the first part, the test on Enzymes is carried out to check the quantity of precipitated calcium carbonate in a test tube. In the second part tests to stabilize sandy soil are performed. The soil samples collected from the bank of river Chenab near the village Ahmalpur were done. Then sieve analysis and hydrometer analysis for the classification of soil were done. After that, the test tube experiments were performed to find the amount of urea, calcium chloride and urease enzymes to be used for better results. Afterward mixing of the sandy soil with a solution of urea, calcium chloride, and urease enzymes in different proportions were done to examine the pH of solutions against different proportions of additives. The additives are, then, mixed with soil in different proportions by different means and modified soil is tested for various outputs. The results obtained from the tests were analyzed and observed for their influence on the performance characteristics of sandy soil. In the end, all the analyzed data is concluded, to compare the results of modified and unmodified sandy soil to study the soil behavior and further recommendations were proposed.

5. LABORATORY TESTING

During the completion of the project, different tests were performed on soil and enzymes. These tests include a sieve analysis test (55) according to ASTM D6913/D6913M–17 standards, hydrometer test (56) according to ASTM D7928−17 standard, direct shear test, permeability test, and Test tube experiment.

5.1 Test-tube Experiment

This series of experiments were performed to analyze the hydrolysis rate of urea which is catalyzed and triggered by urease enzymes and to check the quantity of precipitated calcium carbonate. Various solution of urea-$CaCl_2$ is mixed with urease enzymes to check different quantities of precipitation of calcium carbonate. This test was performed by following the procedure mentioned by Neupane (2013). To perform this test, 10ml of distilled water was taken and various solutions with urea and $CaCl_2$ were made. The solution was shaken gently so that all the chemicals are completely dissolved in water. After proper mixing of urea and calcium chloride, the urease enzyme was added to each solution. The number of urease enzymes was fixed and equal to 0.2g/10ml. After proper mixing, the whole solution was filtered to remove the undissolved particles of enzymes. Figure 1 shows 11 test tubes, five test tubes (T1-T5) for test samples, and other six (C0-C5) i.e., control samples are used. Control samples are used to check whether precipitation is catalyzed by urease enzymes or not.

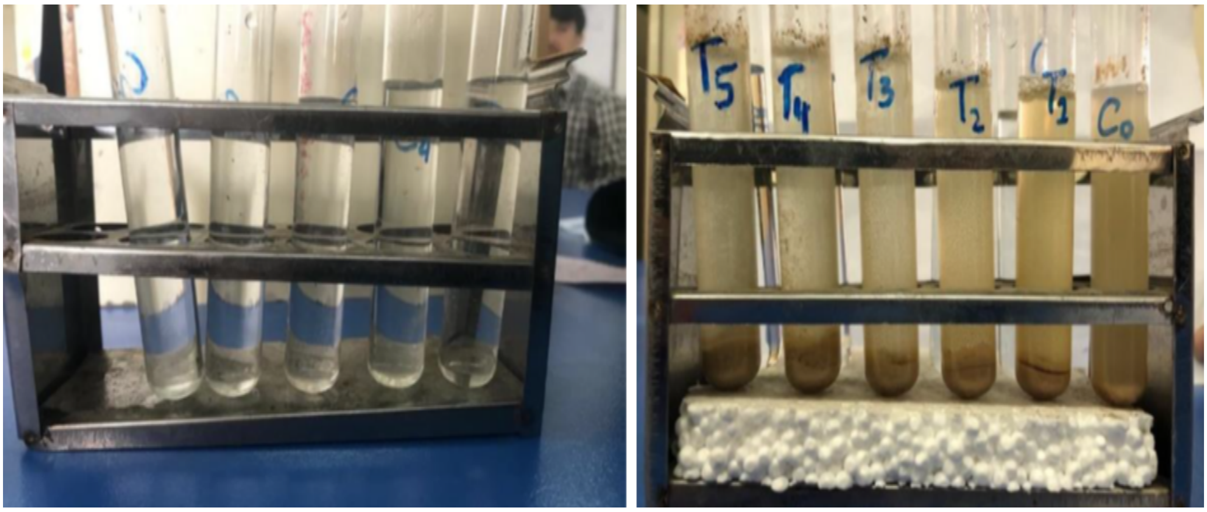

Fig -1: (a) Control Samples; (b) Test samples

5.2 Specific Gravity

The test to find the specific gravity of soil was performed according to ASTM D 854 (57). The test was performed many times using distilled water to ensure the accuracy of the results. The weight of the soil sample taken for this test is 125 grams. The weight of the pycnometer is 197.5g as M1. The weight of the soil and pycnometer is 322.5 grams as M2. The weight of water, soil, and pycnometer is 1270.7 grams as M3. The weight of water added in the pycnometer is 1192.2 grams as M4.

5.3 Direct Shear Test

The consolidated drained shear strength of soil samples under direct shear boundary conditions was found using this test. The specimen of soil is deformed and loaded under a controlled rate on a single shear plane. Three trials on different soil specimens were performed from the same soil sample under different values of normal load to determine the shear strength properties of the soil. The tests are performed according to ASTM D 3080-03 standards (58).

To perform this test, enough sample of soil was used to make four specimens of soil sample with the help of a sampler for each set of tests. In Each set of tests 4 samples are tested against different loading. Water is added to make the sample wet enough to be easily molded. Samples are prepared with the help of a sampler. For untreated soil, two sets of tests were performed. One set of samples is tested just after molding the soil sample. The other set of tests was performed after 14 days of molding samples, to predict the accurate behavior and results of EICP over time as it depends on various factors like soil type and composition, enzyme type and concentration, pH and temperature, stirring rate and intensity. Typically, EICP can take anywhere from a few hours to several days. Figure 2 shows prepared and deformed soil samples for the direct shear test.

Fig -2: (a) Direct shear test samples; (b) Deformed direct shear test samples

For improvement of soil, the soil specimen was mixed with EICP solution by using hands and molded to samples with help of a sampler. The treated soil samples are placed in a safe place and tested after 14 days. After a respective time, the sample was tested using direct shear apparatus. The apparatus was assembled and placed soil specimen was placed in the shear box. The exposed surface of the soil specimen is covered with a porous disk. The whole assembly is then placed in the apparatus and aligned in the loading frame. Then loading is applied to the soil specimen and readings were noted.

5.4 Constant Head Permeability Test

The permeability of soil is a measure of how easily water can flow through the volume of soil. The permeability of soil is a very important factor in geotechnical engineering. Permeability is linked with many mechanical properties of soil. The deformation and strength behavior of soil are linked with the permeability of the soil.

This test was performed by following the standard procedure of ASTM D 2434-68 (59). Firstly, the soil was treated with different EICP solutions. For the improvement of the soil, the EICP solution is added to the soil in a permeameter. The total amount of soil used in each trail is kept constant i.e., 3.655kg. The amount of EICP solutions used in each trial is 600ml. After adding EICP solution to the soil, the soil was left for 14 days to ensure CaCO3 precipitation. After 14 days soil was tested and the coefficient of permeability for each trail are found by constant head permeability test. The volume of oven-dried soil is taken and filled in the permeameter cell such that no gap between soil specimens is visible. The cell is then connected to a water tank and air bubbles are removed. Initially, the soil needed to be fully saturated. For saturation of soil water could enter the cell until water leaves from the other side of the cell. After proper saturation of soil test was performed and different readings were noted like head difference, discharge, and length between manometric tubes. The coefficient of permeability is found by using the equation. Figure 3 shows the injecting process of EICP solution into the soil sample.

Fig -3: Injecting EICP solution into the soil

6. RESULTS AND DISCUSSIONS

This chapter is showing in detail the results and analysis of the tests performed on soil specimens. Results are made for without and with different percentages of EICP solutions. The comparison and analysis of the results are discussed in detail below.

6.1 Soil Classification

Table 1 below shows the particle size of soil and percent passing on different sieve sizes stacked vertically. From the results of sieve analysis (55) and hydrometer analysis (56), the soil is classified, according to a unified classification system, as Silty Sand. Figures 5-7 show the gradation of the soil particles for different trails. The average value of fines present in a soil sample is about 12.32%. In fines, silt particles dominate clayey particles. The average percentage of silt and clay particles are 10.66% and 1.66% respectively.

Table -1: Results of soil classification test showing different percentages of soil type Trails.

Soil Properties	Trial # 01	Trial # 02	Trial # 03
Percentage of sand particles (2mm - 0.075mm)	87.92%	88.18%	86.94%
Percentage of silt particle (0.06mm - 0.002)	10.94%	10.66%	11.94%
Percentage of clay particles (<0.002mm)	1.14%	1.16%	1.12%
D 60	0.2448	0.2404	0.2393
D 30	0.1662	0.1661	0.1653

D 10	0.0608	0.0754	0.0733
Coefficient of Uniformity (Cu)	4.029	3.188	3.2650
Coefficient of Curvature (Cc)	1.857	1.523	1.5572
Percentage of fines	12.08	11.82	13.06

6.2 Test Tube Experiment

This test was performed in accordance with the Neupane et al. (2013) (46) procedure. The purpose of this test was to find the optimum number of components of the EICP solution to be used. Different proportions of urea-$CaCl_2$ were mixed with urease enzymes to find the optimum amount. Different series of tests were performed and are shown below in Table 2. E series were performed to find the best number of enzymes to be used in 10 ml of solution. i.e., control series were performed to check to ensure that without urease enzymes no reaction was seen between urea and $CaCl_2$. T series was performed to find the optimum amount of urea and $CaCl_2$ to be used with the optimum amount of urease enzymes that were found by E series tests in a 10 ml solution. All solutions are left for 14 days and after 14 days precipitation in each tube was observed along with pH values. Table 2-5 shows the number of urease enzymes in the solution and its chemical properties.

Table -2: E series test with different amounts of urease enzymes

Name	Solution (ml)	$CaCl_2$ (g/10ml)	Urea (g/10ml)	Urease Enzymes (g/10ml)
E1	10	1.44	0.6	0.1
E2	10	1.44	0.6	0.2
E3	10	1.44	0.6	0.3
E4	10	1.44	0.6	0.4
E5	10	1.44	0.6	0.5
E11	10	1.08	0.45	0.1
E12	10	1.08	0.45	0.2
E13	10	1.08	0.45	0.3
E14	10	1.08	0.45	0.4
E15	10	1.08	0.45	0.5

Table -3: C and T series test with different amounts of urease enzymes along with PH

Name	Solution (ml)	$CaCl_2$ (g/10ml)	Urea (g/10ml)	Urease Enzymes (g/10ml)	PH
C1	10	0.36	0.15	0	7
C2	10	0.72	0.3	0	6.5
C3	10	1.08	0.45	0	6
C4	10	1.44	0.6	0	5.5
C5	10	1.8	0.75	0	5
C0	10	0	0	0.2	4
T1	10	0.36	0.15	0.2	9
T2	10	0.72	0.3	0.2	8.5
T3	10	1.08	0.45	0.2	7
T4	10	1.44	0.6	0.2	6.5
T5	10	1.8	0.75	0.2	6

Table -4: C and T series test with different amounts of urease enzymes along with PH

Abbreviation	Urease enzymes (g)	Urea (g)	$CaCl_2$ (g)
P1	0.1	0.375	0.9
P2	0.2	0.75	1.8
P3	0.4	1.5	3.6

6.3 Specific Gravity

The test to find the specific gravity of soil was performed according to ASTM D 854 (57) on multiple soil samples the average determined value of the specific gravity of soil is 2.67.

6.4 Direct Shear Test

The direct shear test for the untreated soil tested just after sample molding is shown in Figure 4a, it indicates that the Cohesion value of 10.169 kPa and the angle of friction is 30 degrees. The untreated soil tested just after 14 days of sample molding as shown in Figure 4b, indicates that the Cohesion value of 31.431 kPa and the angle of friction is 30 degrees. The treated soil tested with P1 and tested after 14 days of sample molding is shown in Figure 4c, which indicates that the Cohesion value of 40.675 kPa and the angle of friction is 31 degrees. The treated soil tested with P2 and tested after 14 days of sample molding as shown in Figure 4d, it indicates that the Cohesion value of 43.388 kPa and the angle of friction is 31 degrees. Lastly, the soil treated with P3 and tested after 14 days of sample molding show a Cohesion value of 48.995 kPa and an angle of friction is 30 degrees. It was observed that the soil treated with P3 showed better results in comparison with the soil treated with P2 and P1.

Figure 5 represents the average increase in the mechanical properties of the soil samples under different conditions. Figure 5a shows that the maximum increase in average cohesion value occurs in P3 treated soil sample, which is 48.7 kPa, also the same sample is reported for the increase in average shear strength of 252.1 kPa at failure under the same normal loading conditions in Figure 5b.

6.5 Constant Head Permeability Test

The results of the constant head permeability test represented in Figure 6; it was observed that with an increasing amount of EICP solution the value of the coefficient of permeability decreases significantly which shows an inverse relation. The soil treated with P1, P2, and P3 shows a significant decrease in permeability of the soil. The soil treated with P3 showed better results. To better understand the results, the comparison graph showing the average decrease in permeability of treated soil w.r.t untreated soil is plotted and shown below.

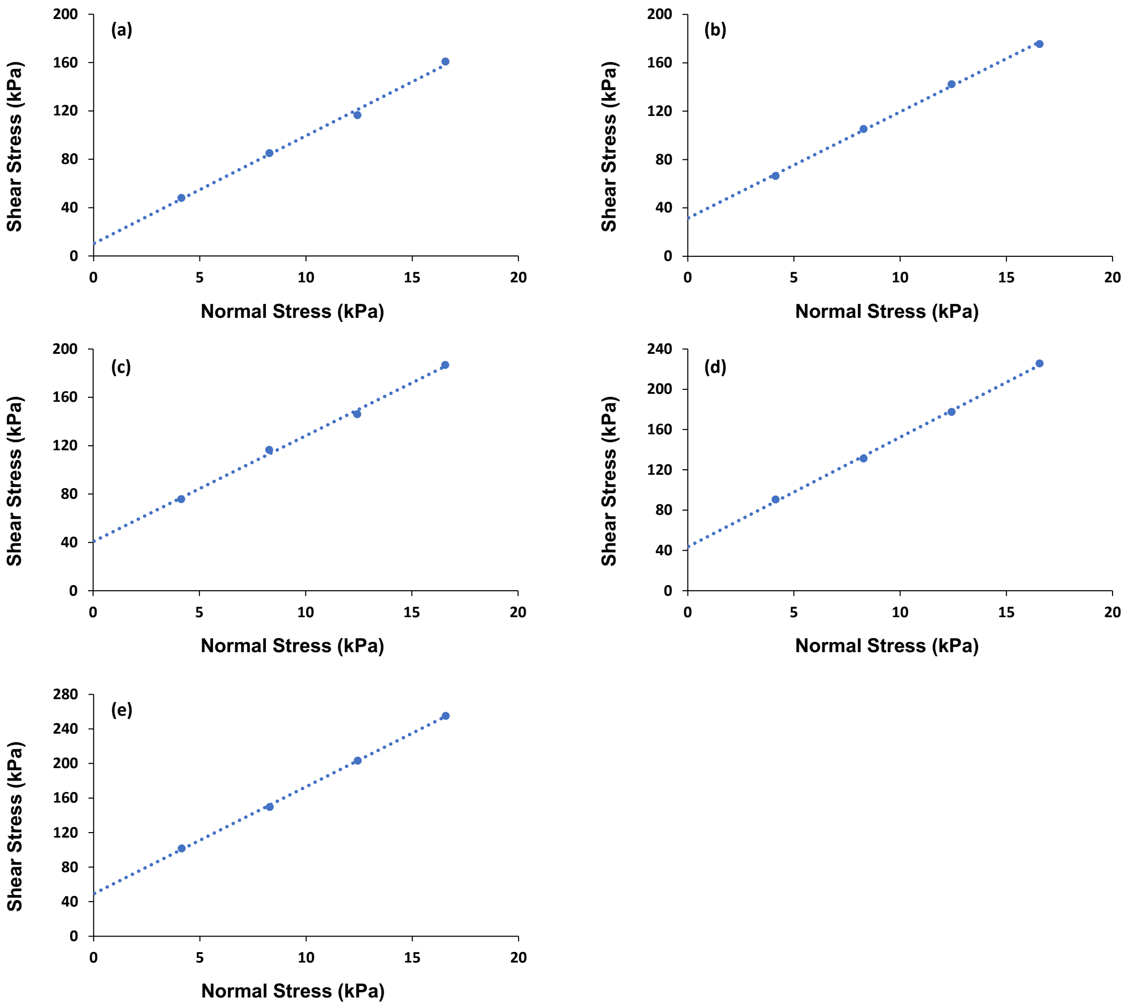

Fig -4: Direct shear test results for (a) untreated soil tested just after molding; (b) untreated soil tested after 14 days of molding; (c) soil treated with P1 and tested after 14 days of molding; (d) soil treated with P2 and tested after 14 days of molding; (e) soil treated with P3 and tested after 14 days of molding

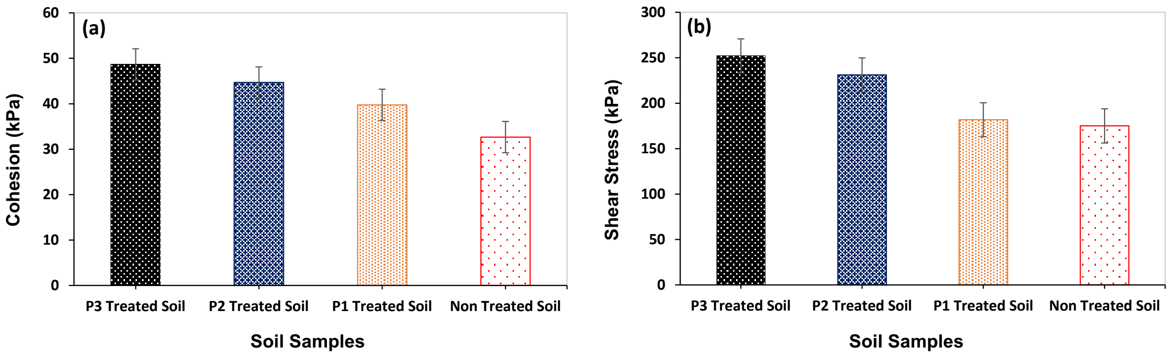

Fig -5: Direct shear test results for the (a) average increase in cohesion (kPa); (b) average increase in shear strength (kPa) at failure under the same normal loading condition

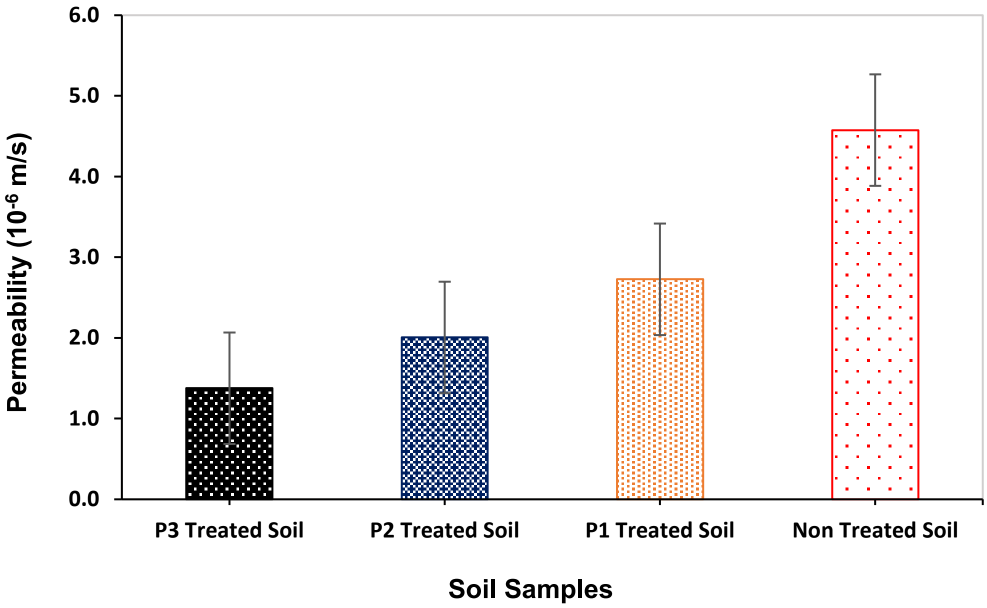

Fig -6: Average increase in the coefficient of permeability (m/s)

7. CONCLUSIONS

Different geotechnical tests were performed to investigate the effect of EICP solution on the mechanical properties of soil i.e., permeability and shear strength of the soil. Tests were performed on both treated soil and non-treated soil. The results are carefully noted and compared. After comparing the results of treated soil and non-treated soil different conclusions are made. The treated soil shows significant improvement in shear strength and permeability of soil as shown in the results.

The results of the experiment on the effects of EICP on soil properties have shown promising results. The EICP treatment has demonstrated improvement in the shear strength of soil by increasing cohesion between soil particles through a process known as bio-cementation. This was evidenced by the 45% improvement in shear strength in soil treated with P3, 32% improvement in soil treated with P2, and a 4% improvement in soil treated with P1. The increase in shear strength is primarily attributed to increased cohesion, with no significant changes in the angle of friction being noted. The shear strength of soil showed a consistent trend of increasing with increasing amounts of EICP solution components.

In addition to the improvement in shear strength, the EICP treatment also reduced the coefficient of permeability of soil through the process of bio-clogging. This was achieved by the precipitation of calcium carbonate crystals, which filled the voids in the soil, thereby increasing resistance to the flow of water. The soil treated with P3 showed a 70% reduction in permeability, soil treated with P2 showed a 50% reduction, and soil treated with P1 showed a 40% reduction. The permeability of soil also showed a decreasing trend with increasing amounts of EICP solution components.

In conclusion, the experimental results provide evidence for the effectiveness of EICP in the field of Geotech. The improvement in shear strength and reduction in permeability of soil demonstrate the potential of EICP to provide effective solutions for soil stabilization and improve soil structure in various geotechnical applications.

8. RECOMMENDATIONS

Since this was the start of using EICP in improving the geotechnical properties of soil. We have worked on the shear strength and hydraulic conductivity of soil, and commercially purchased enzymes were used for this study. We would like to recommend some further studies in this regard.

- Instead of using commercially purchased urease enzymes, enzymes extracted from different naturally found resources like jack bean and watermelon seeds, etc. will be used with soil to check their behavior in the geotechnical properties of soil.
- The effect of EICP solutions on different geotechnical properties is still a mystery.
- Since the density of soil affects the geotechnical properties since this study was done with a specific single value of density. So, using different values of EICP solutions at different sets of densities is also an interesting field of research in this regard.

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