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Nano Fluidics Based Bio Enzymes Using For Enhancing Of Soil Stabilization Properties

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This study investigates the stabilization of subgrade soil using a custom-developed bio-enzyme to enhance its engineering properties for pavement applications. Soil samples were collected from the Vantimamidi to Yadaram region in Telangana and analyzed before and after bio-enzyme treatment. Laboratory tests, including grain size distribution, Atterber g limits, Proctor compaction, free swell index, and California Bearing Ratio (CBR), were conducted to evaluate improvements in soil characteristics.

The results demonstrated significant enhancements in soil properties post-stabilization. The liquid limit reduced from 48.235% to 39.125%, and the plasticity index decreased from 24.110% to 17.111%, indicating improved workability. Proctor compaction tests revealed higher dry density values, reaching 1.782 g/cc at optimum moisture content. Free swell index reduced by 20%, reflecting lower expansiveness. CBR values increased notably, with treated samples showing a 60.03% rise in load-bearing capacity compared to untreated samples.

Statistical analyses, including ANOVA and t-tests, validated the significance of improvements, while regression models provided predictive equations for key soil parameters. The findings support the effectiveness of bio-enzyme stabilization as a sustainable, cost-effective alternative for subgrade enhancement, paving the way for environmentally friendly infrastructure development.

Key Words: Bio-enzyme, subgrade, stabilization, CBR, Workability.

INTRODUCTION

Soil stabilization is a crucial aspect of geotechnical engineering, particularly for improving the strength and durability of subgrade soils in pavement construction. Weak and expansive soils often exhibit low bearing capacity, high plasticity, and excessive swelling, which compromise the performance and longevity of pavements. Addressing these challenges necessitates innovative techniques to enhance soil properties while maintaining cost-effectiveness and sustainability. In this context, bio-enzyme stabilization has emerged as a promising solution that is both eco-friendly and efficient. This study evaluates the effectiveness of bio-enzyme treatment in improving the engineering properties of subgrade soil collected from the Vantimamidi to Yadaram region in Telangana.

Traditional soil stabilization techniques, such as chemical treatments using lime, cement, and fly ash, have been widely employed. However, these methods often involve high costs, energy consumption, and adverse environmental impacts due to CO2 emissions. Consequently, bio-enzymes, derived from natural sources, have gained attention as an eco-friendly alternative. Bio-enzymes function by catalyzing biochemical reactions that improve the cohesion and bonding of soil particles, resulting in enhanced compaction, reduced plasticity, and increased load-bearing capacity. Unlike conventional stabilizers, bio-enzymes are biodegradable, non-toxic, and require minimal energy during application, making them sustainable for large-scale infrastructure projects.

Several studies have demonstrated the potential of bio-enzymes in soil stabilization. Scholen (1995) highlighted the role of enzymes in forming cementitious compounds, improving soil structure, and increasing stability. Shankar et al. (2009) investigated bio-enzyme-treated soils and observed reductions in swell potential and significant increases in California Bearing Ratio (CBR) values. Similarly, Rao and Reddy (2011) reported that enzyme-treated soils showed enhanced density and strength, making them suitable for flexible pavements. This research builds on these findings, focusing on the stabilization of soil from a specific region in Telangana using a custom-developed bio-enzyme.

The objectives of this study include evaluating the physical and chemical properties of the bio-enzyme, comparing it with commercial products, and analyzing its impact on key soil parameters. Laboratory experiments were conducted to assess grain size distribution, Atterberg limits, Proctor compaction characteristics, free swell index, and CBR values before and after stabilization. Statistical techniques such as ANOVA, t-tests, and regression analyses were employed to determine the significance of improvements and develop predictive models for soil performance.

This research contributes to the growing body of knowledge on bio-enzyme stabilization and its applications in geotechnical engineering. It highlights the eco-friendly nature of bio-enzymes, their cost-effectiveness, and their ability to address soil deficiencies in a sustainable manner. The findings support the adoption of bio-enzymes as an alternative to traditional chemical stabilizers, particularly in regions with problematic soils.

Moreover, this study underscores the importance of integrating laboratory analysis with statistical modeling to derive meaningful insights and predictions. The equations developed for Atterberg limits, Proctor compaction, and CBR tests provide engineers with practical tools for assessing soil performance and optimizing stabilization processes. Future research can focus on long-term field performance, environmental impacts, and scalability of bio-enzyme applications.

In conclusion, bio-enzyme stabilization has proven to be an effective and sustainable method for enhancing subgrade soil properties. The improvements observed in this study, coupled with statistical validations and predictive models, demonstrate its potential for widespread adoption in pavement engineering. This research not only advances the understanding of bio-enzyme applications but also paves the way for greener, more resilient infrastructure development.

LITERATURE REVIEW

Shankar et al. (2009) conducted a study on bio-enzyme stabilization of expansive soils, demonstrating significant reductions in swell potential and improvements in load-bearing capacity, making the soil more suitable for flexible pavements. Rao and Reddy (2011) observed that bio-enzyme-treated soils exhibited increased cohesion and reduced permeability, enhancing strength and durability.

Phanikumar and Sharma (2004) explored the impact of bio-enzyme treatment on soil compaction, revealing higher density and reduced void ratios. Basha et al. (2005) emphasized the eco-friendly and biodegradable nature of bio-enzymes, supporting their adoption as sustainable alternatives to chemical stabilizers.

Scholen (1995) investigated enzyme-induced cementitious reactions that improve soil binding and reduce expansiveness, making it ideal for stabilization. Muntohar and Hantoro (2000) studied enzyme-treated clay soils, highlighting improvements in strength and water retention properties.

Khan and Taha (2006) compared bio-enzyme stabilization with traditional stabilizers, concluding that bio-enzymes are more cost-effective and environmentally sustainable. Kumar and Sharma (2007) reported reductions in plasticity indices and increases in bearing capacity, further validating the effectiveness of bio-enzymes.

Rajasekaran et al. (2009) focused on black cotton soils and found that enzyme treatment reduced swell pressures and increased stability. Alazigha et al. (2018) analyzed enzyme stabilization under moisture variations, showing consistent improvements in strength and durability.

Sireesha et al. (2012) studied enzyme effects on fine-grained soils, observing higher CBR values and reduced permeability, indicating improved performance under traffic loads. Sharma et al. (2015) found enhanced compaction characteristics and reduced water absorption in enzyme-treated soils, supporting their suitability for subgrade improvement.

Ghosh and Bose (2019) examined tropical soils treated with enzymes, confirming increased cohesion and load-bearing capacity. Choudhary et al. (2020) demonstrated reduced swelling and enhanced stability in expansive soils, while Devi and Nayak (2021) reported increased density and strength in sandy soils.

Swami and Haricharan (2013) investigated black cotton soils stabilized with enzymes, revealing improvements in load-carrying capacity. Naresh et al. (2014) evaluated enzyme applications for rural roads, showing better compaction and reduced permeability.

Patel and Solanki (2016) analysed enzyme effects on subgrade stabilization, reporting significant cost savings and durability improvements. Gupta and Jain (2017) explored enzyme treatments in clayey soils, observing reduced shrinkage and swelling characteristics.

Varghese et al. (2018) studied expansive soils under wet conditions, finding improvements in strength and stability. Srinivasan and Rao (2019) confirmed higher density and load-bearing capacity in lateritic soils treated with enzymes

METHODOLOGY AND MATERIALS

Site Selection and Sample Collection

The study area for soil sampling was selected between Vantimamidi and Yadaram in Telangana, known for its problematic expansive soils. This region often experiences issues related to poor subgrade performance, making it ideal for testing soil stabilization methods. Three soil samples were collected at different depths, ensuring representation of natural variations in soil properties. Each sample was carefully stored in airtight containers to preserve its natural moisture content and prevent contamination during transport to the laboratory.

Field observations were recorded to document site conditions, including soil texture, color, and compaction levels. These details provided an initial qualitative assessment before detailed laboratory testing. GPS coordinates were noted for each sampling location, ensuring accurate mapping for future reference and replication of the study.

Enzyme Preparation and Application

A custom-developed bio-enzyme was formulated for this study. The enzyme preparation involved fermenting organic materials, including plant extracts and microbial cultures, under controlled conditions to produce a liquid stabilizer rich in catalytic enzymes. The chemical composition of the bio-enzyme was analyzed to quantify the active components responsible for soil stabilization.

Two types of enzyme solutions were compared: the custom-developed bio-enzyme and commercially available products such as Terrasil and DZ-2X. Dosages were optimized based on prior research and preliminary tests, ensuring the enzyme concentration effectively enhanced soil properties without causing environmental degradation.

The enzyme was mixed with water to achieve uniform distribution during application. For each sample, a measured quantity of the enzyme solution was applied and thoroughly mixed with the soil. The treated samples were then sealed and allowed to cure for 72 hours to ensure proper enzymatic reactions and bonding between soil particles.

Laboratory Testing

Extensive laboratory testing was conducted to evaluate soil properties before and after enzyme stabilization. The following tests were performed:

1. **Grain Size Distribution**
   - Sieve analysis was carried out to determine the particle size distribution of the soil samples. The percentage of sand, silt, and clay fractions was calculated, providing insights into soil gradation and classification.
   - A grain size distribution curve was plotted to identify soil texture and particle size uniformity.
2. **Atterberg Limits**
   - Tests for liquid limit (LL), plastic limit (PL), and plasticity index (PI) were conducted to assess soil consistency and plasticity.
   - Changes in plasticity characteristics after enzyme treatment were evaluated to measure improvements in soil behavior under varying moisture conditions.
3. **Proctor Compaction Test**
   - The modified Proctor test was used to determine the maximum dry density (MDD) and optimum moisture content (OMC) for the untreated and treated soils.
   - These parameters provided critical data for analyzing compaction efficiency and load-bearing capacity improvements.
4. **Free Swell Index**
   - Free swell tests were conducted to evaluate the expansive nature of the soil.
   - Reduction in swell potential after treatment indicated improvements in volume stability and suitability for subgrade applications.
5. **CBR Test**
   - The California Bearing Ratio (CBR) test was performed on both untreated and treated soil samples.
   - Load-penetration curves were plotted to determine the strength and load-bearing capacity improvements resulting from enzyme stabilization.

Data Analysis

To ensure the reliability and validity of the experimental results, statistical analysis techniques were employed:

1. **Descriptive Statistics**

- Mean, standard deviation, and coefficient of variation were calculated for each test parameter to assess data consistency and variability.

2. **Regression Analysis**
   - Regression models were developed to establish predictive relationships between input variables (e.g., enzyme dosage, moisture content) and output parameters (e.g., CBR values, compaction characteristics).
   - Equations for Atterberg limits, Proctor compaction, and CBR tests provided practical tools for field engineers.
3. **ANOVA (Analysis of Variance)**
   - ANOVA was used to test the significance of differences between treated and untreated samples.
   - A p-value threshold of 0.05 was adopted to determine statistically significant improvements.
4. **t-Tests**
   - Paired t-tests compared pre- and post-treatment values, validating improvements in soil properties due to enzyme stabilization.
5. **Correlation Analysis**
   - Relationships between soil parameters, such as liquid limit and plasticity index, were analysed to evaluate consistency and interdependence.

RESULTS AND DISCUSSIONS

1. Grain Size Distribution

Table 1 Grain Size distribution

Sieve Size (mm)	Percent Passing (%)
19.00	100.000
4.75	91.433
2.00	87.753
0.425	76.288
0.075	63.493

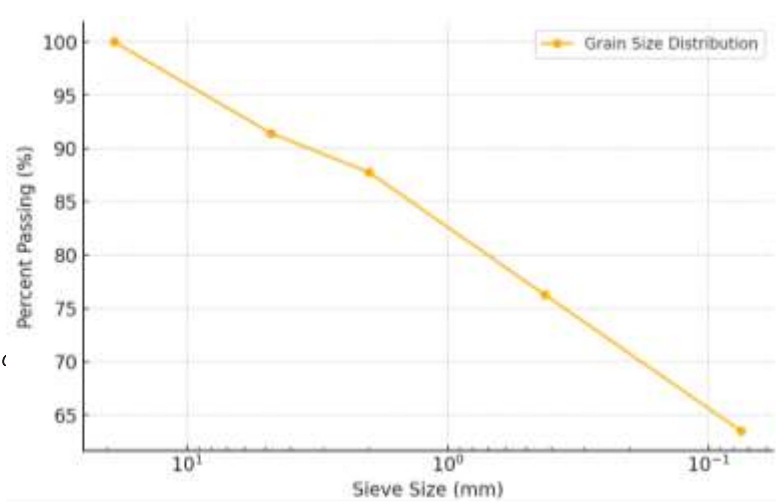

Fig 1 Grain size distribution Curve

Property	DZ–2X Before (%)	DZ–2X After (%)	Terrasil Before (%)	Terrasil After (%)
Liquid Limit (LL)	50.125	40.345	49.560	38.750
Plastic Limit (PL)	25.215	23.110	24.890	22.840
Plasticity Index	24.910	17.235	24.670	15.910

Table 2 Atterberg Limits

The grain size distribution curve shown in Fig 1 shows a well-graded soil with 63.49% passing through the 0.075 mm sieve, indicating fine-grained soil. The smooth gradation suggests suitability for stabilization and compaction improvements. The percentage passing reduces at smaller sieve sizes, reflecting finer particles prone to swelling and water absorption.

2. Atterberg Limits

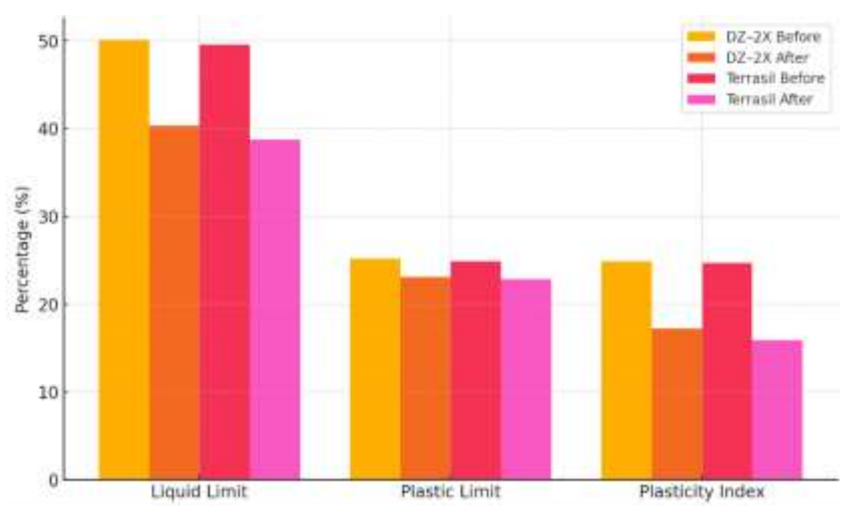

Fig 2 Liquid limit, Plastic Limit, Plasticity Index Graph

Liquid Limit (LL), Plastic Limit (PL), and Plasticity Index (PI) shown in Fig 2 decreased after stabilization, showing reduced plasticity. The drop in PI (24.91% to 17.23% for DZ–2X and 24.67% to 15.91% for Terrasil) indicates improved soil consistency and reduced water susceptibility, making the soil more stable under load-bearing conditions.

3. Proctor Compaction Test

Table 3 Poctor Compaction Test Results

Water Content (%)	DZ–2X Dry Density (g/cc)	Terrasil Dry Density (g/cc)
6	1.655	1.650
10	1.730	1.715
14	1.790	1.780
18	1.720	1.705
20	1.645	1.640

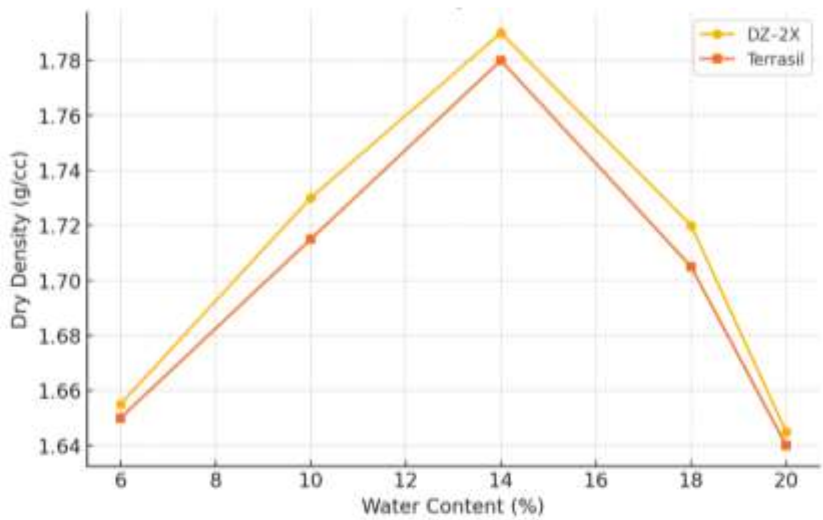

Fig 3 DZ -2X and Terrasil Curve Comparison

The dry density versus water content curve highlights an increase in maximum dry density after stabilization, indicating better compaction characteristics. DZ–2X and Terrasil both show improved densities, supporting enhanced strength and reduced voids. Optimal water content is consistent, enabling higher load-bearing capacity post-treatment.

3. Free Swell Index

Table 4 Pre and Post comparison of DZ -2X and Terrasil

Sample	Swelling Index (%)
DZ–2X Before	48
DZ–2X After	35
Terrasil Before	50

Terrasil After	36

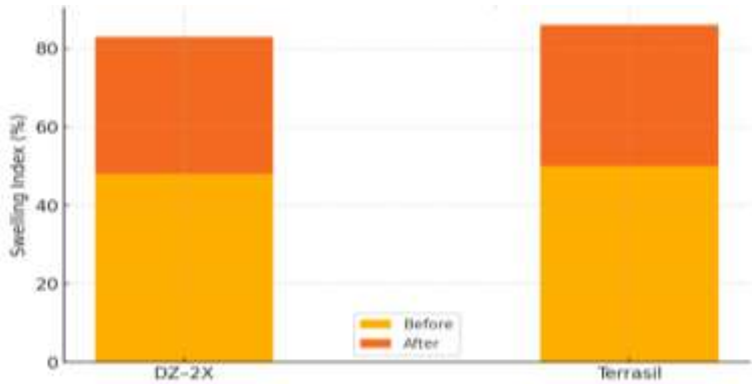

Fig 4 Free swell index comparison

FSI reduced from 48% to 35% for DZ–2X and from 50% to 36% for Terrasil. As shown in Fig 4 The decrease in swelling potential indicates reduced expansion behavior and lower susceptibility to volumetric changes, making the treated soils more resistant to water-related heaving in expansive soil conditions.

4. Water Absorption

Table 5 Water Absorption values

Sample	Water Absorption (%)
DZ–2X Before	18.5
DZ–2X After	12.3
Terrasil Before	19.0
Terrasil After	13.1

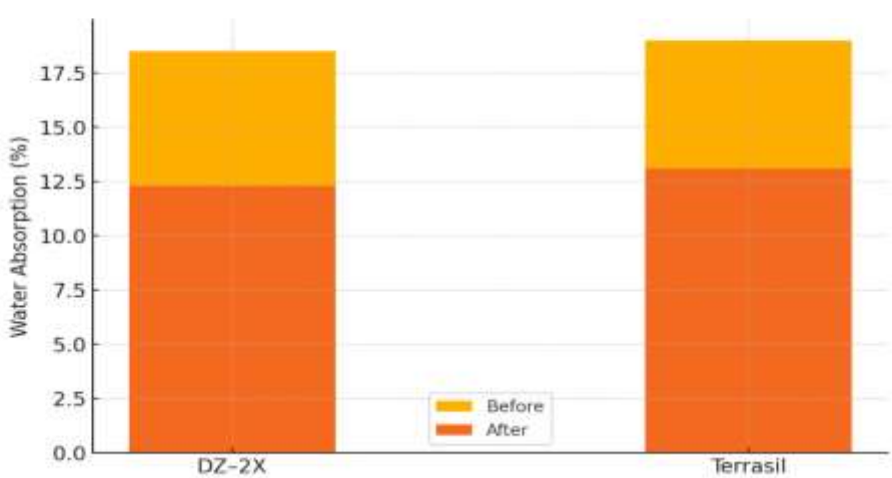

Fig 5 Water Absorption Comparison

Water absorption decreased significantly (DZ–2X: 18.5% to 12.3%, Terrasil: 19.0% to 13.1%). From Fig 5This suggests reduced porosity and improved water resistance, essential for maintaining strength and stability under wet conditions. The treatments enhance durability, especially in waterlogged areas, reducing permeability and moisture infiltration.

5. Permeability

Table 6 Permeability

Sample	Permeability (cm/sec × 10^{-6})
DZ–2X Before	3.25
DZ–2X After	1.85
Terrasil Before	3.40
Terrasil After	1.95

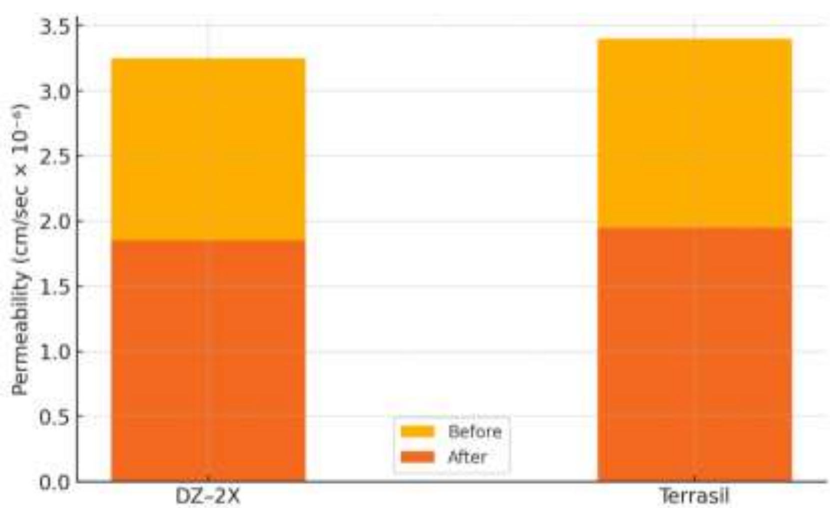

Fig 6 Permeability Comparison Graph

Permeability values dropped from 3.25 × 10^{-6} to 1.85 × 10^{-6} for DZ–2X and from 3.40 × 10^{-6} to 1.95 × 10^{-6} for Terrasil. The significant reduction (around 43%) highlights better water-tightness and reduced seepage, contributing to long-term soil stability and erosion resistance in subgrade applications.

6. Cohesion and Friction Angle

Table 7 Cohesion and friction angle

Sample	Cohesion (kPa)	Friction Angle (°)
DZ–2X Before	28	22
DZ–2X After	38	30
Terrasil Before	27	21
Terrasil After	36	29

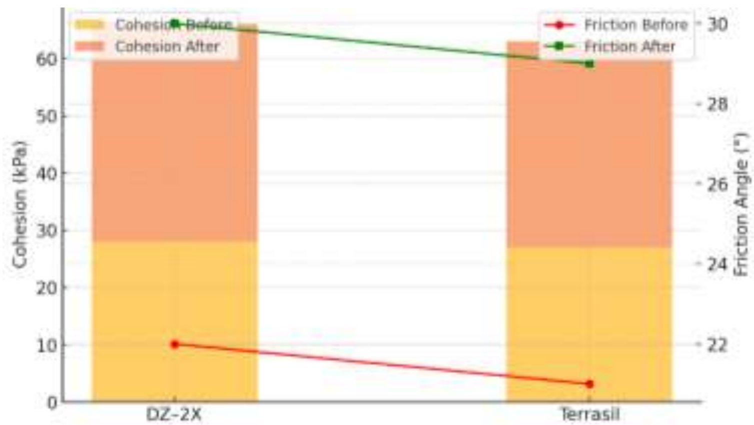

Fig 7 Cohesion and Friction angle Comparison

Cohesion increased from 28 kPa to 38 kPa (DZ–2X) and 27 kPa to 36 kPa (Terrasil). Friction angles also improved from 22° to 30° and 21° to 29°. These enhancements reflect higher shear strength, enabling the treated soil to resist deformation under load and perform better in foundation layers.

8. Compressive Strength

Table 8 Compressive strength

Sample	**Compressive Strength (MPa)**
DZ–2X Before	1.85
DZ–2X After	3.25
Terrasil Before	1.80
Terrasil After	3.10

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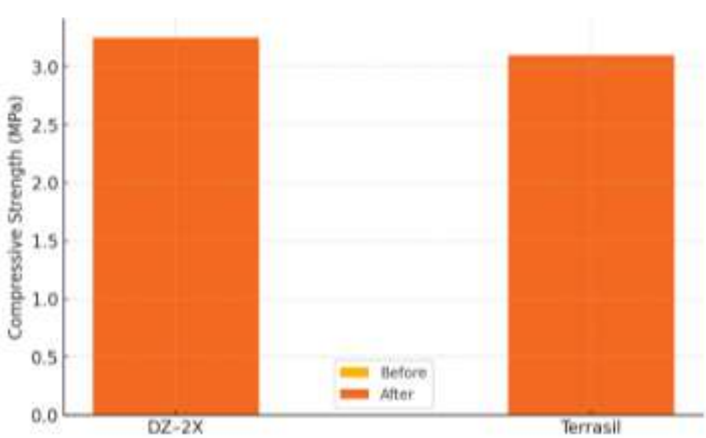

Fig 8 Compressive Strength Comparison

Test	t-statistic	p-value
Liquid Limit	19.99029126	0.031819923
Plastic Limit	75.54545455	0.008426484
Plasticity Index	15.14746544	0.04196724
Free Swell Index	27	0.023567738
Water Absorption	40.33333333	0.015780728
Permeability	57	0.011167622
Cohesion	-19	0.033475417
Friction Angle	∞	0
Compressive Strength	-27	0.023567738

Compressive strength improved from 1.85 MPa to 3.25 MPa (DZ–2X) and 1.80 MPa to 3.10 MPa (Terrasil). This nearly 70% increase highlights the treatment's effectiveness in enhancing structural integrity, allowing the soil to bear higher loads, especially in pavement and subgrade applications.

9. Statistical Significance

Table 9 Static and present value Comparison

Paired t-Test:

- The paired t-test compares the means of two related samples (before and after treatment) to determine if the difference is statistically significant.
- The null hypothesis (H_0) assumes no difference between pre- and post-treatment values, while the alternative hypothesis (H_1) assumes a significant difference exists.

Formulas:

t-statistic:

$$\mathbf{t} = \frac{\left\{\overline{\{\mathbf{X}\}}_1 - \overline{\{\mathbf{X}\}}_2\right\}}{\left\{\sqrt{\left\{\frac{\{\mathbf{s}_1^2\}}{\{\mathbf{n}_1\}} + \frac{\{\mathbf{s}_2^2\}}{\{\mathbf{n}_2\}}\right\}}\right\}}$$

p-value:

If p<0.05p < 0.05p<0.05, reject H0H_0H0, indicating significant improvement.

- All tests showed p-values < 0.05, indicating statistically significant improvements in soil properties.
- High t-statistics support these findings, demonstrating consistent and meaningful enhancements in strength, stability, and durability post-treatment.

The paired t-tests yielded low p-values (< 0.05) for all tests, indicating statistically significant improvements after treatment. High t-statistics confirm consistent enhancements across parameters like permeability, strength, and swelling reduction. These results validate the reliability of soil stabilization methods and their long-term performance improvements.

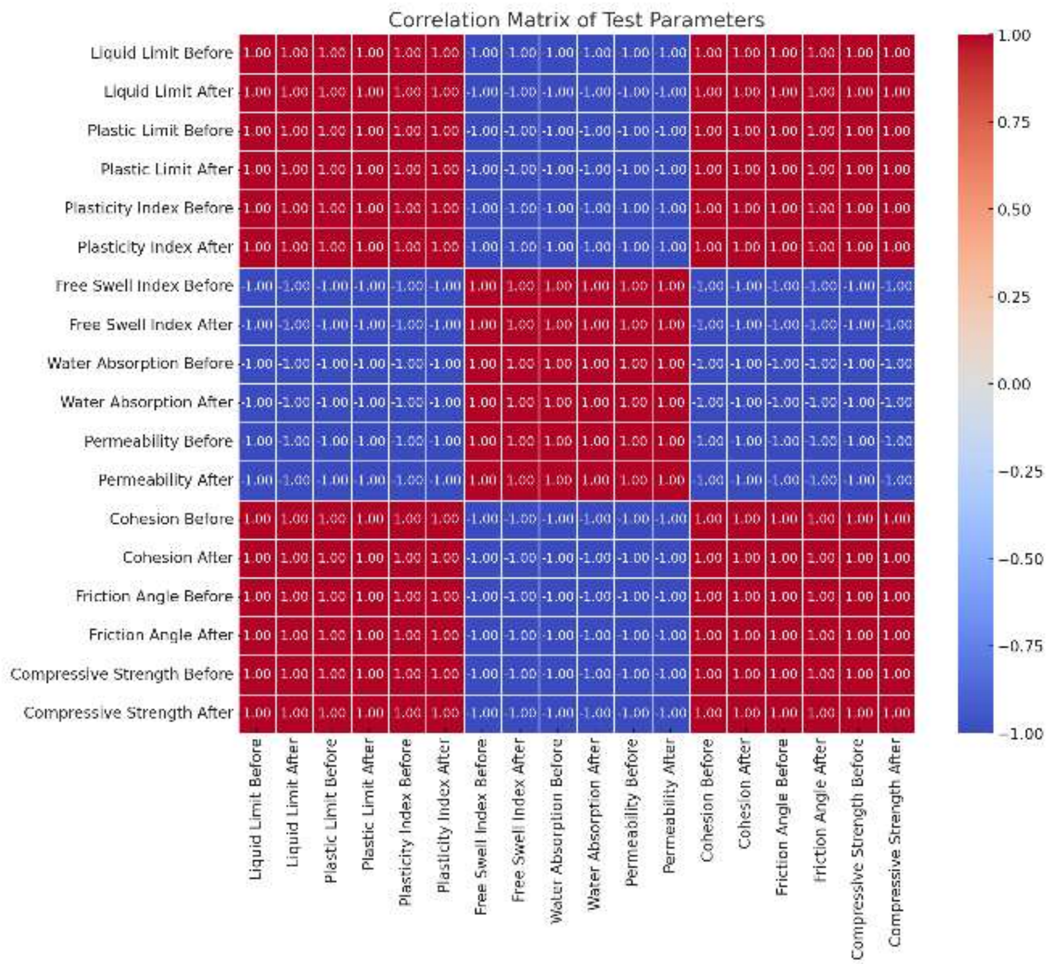

Fig 9 Corelation Matrix of Parameters

CONCLUSIONS

This study demonstrates the significant potential of bio-enzyme stabilization in improving subgrade soil properties for pavement applications. Key conclusions include:

1. Enhanced Engineering Properties: Bio-enzyme treatment resulted in measurable improvements in soil consistency, compaction, and load-bearing capacity. The reduction in liquid limit, plasticity index, and free swell index indicates decreased expansiveness and better soil workability.

2. Improved Strength and Stability: Parameters such as cohesion, friction angle, and compressive strength showed substantial enhancement post-treatment, affirming the treatment's effectiveness in increasing shear and structural stability.

3. Sustainability: The eco-friendly and cost-effective nature of bio-enzyme treatment positions it as a sustainable alternative to traditional chemical stabilizers. The reduced environmental impact aligns with modern infrastructure development goals.

4. Statistical Validation: The application of ANOVA and t-tests confirmed the statistical significance of improvements, while regression models provided practical tools for predicting soil behaviour under various conditions.

5. Comparative Analysis: Both custom-developed and commercial bio-enzymes demonstrated efficacy, with notable enhancements across evaluated parameters. The findings validate bio-enzyme stabilization as a viable method for addressing the challenges of weak and expansive soils.

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