## Heliyon 6 (2020) e03384

Contents lists available at ScienceDirect

# Heliyon

journal homepage: www.cell.com/heliyon

**Research article** 

# Improving the characteristics of dispersive subgrade soils using lime

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### ARTICLE INFO

Keywords:

Subgrade

Lime

Stabilization

Improvement

Dispersive soil

Civil engineering

ABSTRACT

Dispersive soil arises significant problems that need attention in geotechnical Engineering. Such soils are easily erodible and keep apart due to the difference in moisture content and exchangeable sodium. This study focuses on enhancing sub-grade of the road by stabilizing dispersive soil with lime, and it provides better index properties, reduces dispersivity, increases Unconfined compression strength, and California Bearing Ratio value with an increasing lime quantity and curing in different test conditions. The effective lime content should be 7% to 9 % of dry soil weight as it provides high strength and quality of subgrade pavement rating.

#### 1. Introduction

The soils that are highly susceptible to erosion and containing a high percentage of exchangeable sodium ions are called Dispersive soils by (Sivapullaiah et al., 2000). In such soils, the peculiar phenomenon happens with an increment in moisture content that sometimes inflicts significant damages on construction projects. The Physico-chemical characteristics of the particles in dispersive soils cause them to disperse and separate from one another upon the mixing of water. If dispersive clays are not accurately identified, they may lead to catastrophic damages and failures in any project. This kind of soil is readily eroded and exhibit low-level stress conditions and low hydraulic gradient by (Foster et al., 2000). Chemical stabilization is applied as a cost-effective, environmentally friendly, and efficient method for soil treatment. It is also well known that stabilizing soil with local natural, industrial resources, mainly lime, cement, and fly ash has a significant effect on improving the soil properties (Harichane et al., 2001). Calcium hydroxide (slaked lime) is most widely used for stabilization. Calcium oxide (quick lime) may be more effective in some cases; however, the quick lime will corrosively attack equipment and may cause severe skin burns to personnel. Laboratory testing indicated that slaked lime reacts with medium, moderately fine, and fine-grained soils to produce decreased plasticity, increased workability, and increased strength (Little, 1995). Strength gain is primarily due to the chemical reactions that occur between the lime and soil particles. These chemical reactions occur in two phases, with both immediate and long-term benefits. The process by which the characteristics of the soil are enhanced to meet the construction requirement is called stabilization. In its broadest sense, soil stabilization may also be defined

as a method used to change a few characteristics of the soil to improve the desired performance of the soil. A significant level of long-term strength improvement in lime stabilized soils and aggregates is possible and probable (Dallas, 1999). The traditional lime stabilization can be defined as lime mixed into the soil and immediately compacted without allowing the lime/soil mixture to sit for an extended period before compaction (Harris et al., 2004). Therefore, the addition of lime can significantly improve pozzolanic strength and reduction in plasticity (Little, 1999) and an apparent reduction in clay content with an increase in the percentage of coarse particles (Kumar et al., 2012). Lime Stabilized soil has been used to upgrade both handling and mechanical purposes in civil engineering work (Sherwood, 1993). The degree of stabilization relies on soil-lime reaction. The major influences of this reaction are an increase in shear strength, bearing capacity, and a decrease in the dispersivity of the soils.

A double hydrometer test, also called the Soil Conservation Service laboratory dispersion test, is one of the primary methods adopted to examine the dispersion of clay soils by (Volk, 1937). The standard hydrometer test provides particle size distribution in which soil sample dissolves in distilled water with sodium Hexametaphosphate, and a parallel hydrometer test is then done on a similar soil sample but without chemical dispersant. The dispersion percentage is the ratio of the dry mass (particle smaller than 0.005mm diameter) in a test without dispersing agent to the dry mass (particle smaller than 0.005mm diameter) with a dispersing agent. Procedures for performing the test are outlined in USBR 5405 (U.S. Department of the Interior, 1991). Table 1 shows the result of a double hydrometer test evaluating the degree of dispersion observed by (Sherard and Decker, 1977).

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https://doi.org/10.1016/j.heliyon.2020.e03384

Received 5 April 2019; Received in revised form 11 November 2019; Accepted 4 February 2020

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# Table 1. Determination of dispersion degree from double hydrometer test.

Percent dispersion	Degree of dispersion
<30	Non-dispersive
30 to 50	Intermediate
>50	Dispersive

Table 2. Dispersion of Untreated Subgrade Dispersive soil.							
Sample (pits)	P-1	P-2	P-3	P-4	P-5		
Dispersion (%)	54.7	69.2	58.8	66.3	48.8		



Figure 1. Double Hydrometer test of Dispersive soil.



Figure 2. View of conducting a double Hydrometer Test on dispersive soil.

The concentration of different ion was estimated from soil pore water chemical test results. There is a dependency between the electrolyte concentration of the soil pore water and exchangeable ions present. The existence of a high amount of sodium concentration makes the soil more dispersive. Therefore, Sodium absorption Ratio (SAR) and Percent Sodium (PS) are the two parameters used to check chemical compatibility (Lashkaripour and Soloki, 2003)

# $SAR = \frac{Na}{0.5(Ca + Mg)} \quad \text{with units of } meq/L \tag{1}$

All soils were dispersive if SAR exceeded 2.

$$PS = \frac{Na(100)}{TDS} \quad with \ TDS = Ca + Mg + Na + K$$
(2)

With all units in meq/L of saturation extract. Soils were dispersive if PS exceeded 60%

The main chiesting of this study is to identify on

The main objective of this study is to identify and improve the performance of dispersive soil when stabilized with hydrated (slaked) lime for improving sub-grade.

### 2. Materials and experimental methods

Characterization of the primary materials utilized in this study is essential to predict the necessary behavior of treated soil. This includes hydrated lime (Ca (OH)  $_2$ ) and soil. The primary raw material for the production of lime is limestone. In the current study, hydrated lime (Ca (OH)  $_2$ ) was used from a blue star reagent and equipment distributor from Addis Abeba, Ethiopia. The study deals with the stabilization of a soil called dispersive soil that was found in the Southern Nations, Nationalities, and Peoples Regional State (SNNPRS) in the Arba Minch area and Derashe Woreda, Ethiopia. It spreads below a depth of 1.0 m from the ground level and extends to depths more than 10 m. It has sufficient strength in a dry situation, but it loses strength as moisture content increases. Failures were observed on the road surface where this soil is present.

The soil was collected from the above-mentioned area as the primary material for the present research work. It was stabilized with hydrated lime. Soil characterization was necessary for establishing the effect of Lime stabilization, and therefore, it was applied by performing various tests described in the next sections. Five test pits were made abbreviated as p-1, p-2, p-3, p-4, and p-5 for simplification. This soil was Clayey with silt material and was regarded as troublous under wet conditions.

# 3. Results and discussions

The study deals with how lime usage could enhance the geotechnical characteristics [including consistency limits, grain size distributions properties, compaction properties, unconfined compressive strength, Dispersive properties, Chemical property, durability and California bearing ratio (CBR)] of dispersive subgrade soil collected from Wozeka Gidole road project.

# 3.1. Double hydrometer test for the determination of dispersion

The dispersion percentage is the ratio of the dry mass (particle smaller than 0.005mm diameter) in a test without dispersing agent to the dry mass (particle smaller than 0.005mm diameter) with a dispersing agent. Table 2 shows a high percentage of soils with dispersive characteristics, exhibited dispersion when tested by a Double hydrometer test. Figure 1 shows the untreated dispersive soil with a standard hydrometer and parallel hydrometer test with an identical soil specimen, but without chemical dispersant.

Table 3. Exchange	able 3. Exchangeable cations in untreated soil samples.						
Test Pit No.	Ca <sup>++</sup>	$Mg^{++}$	$Na^+$	$\mathbf{K}^+$	SAR	PS	Description
P-1	0.44	0.26	5.54	2.68	15.8	62.11	Dispersive
P-2	0.56	0.31	6.21	2.10	14.3	67.65	Dispersive
P-3	0.49	0.28	5.51	1.89	14.31	67.44	Dispersive
P-4	0.42	0.21	6.40	2.30	20.32	70.1	Dispersive
P-5	0.39	0.18	5.40	2.80	18.94	61.6	Dispersive

# Table 4. Classification of Untreated Subgrade soil.

P-1	P-2	P-3	P-4	P-5
100	100	100	100	100
94.6	95.2	98.6	92.6	95.8
82.4	70.6	84.2	82	86.2
29.03	34.67	38.57	31.68	26.08
2.7	2.72	2.74	2.75	2.76
0	0	0	0	0
17.6	29.4	15.8	18	13.8
43.75	29.39	20.1	35	38.79
38.65	41.21	64.1	47	47.41
75.03	76.98	67.88	67.41	60.57
31.97	32.39	33.19	31.89	16.72
A-7-5	A-7-5	A-7-5	A-7-5	A-7-5
31.61	26.15	32.74	30.51	20.3
MH	MH	CH	CH	MH
Clayey and Silt Material				
54.7	69.2	58.8	66.3	48.8
15.8	14.3	14.31	20.32	18.94
62.11	67.65	67.44	70.1	61.6
	P-1   100   94.6   82.4   29.03   2.7   0   17.6   43.75   38.65   75.03   31.97   A-7-5   31.61   MH   Clayey and Silt Material   54.7   15.8   62.11	P-1 P-2   100 100   94.6 95.2   82.4 70.6   29.03 34.67   29.03 34.67   27 2.72   0 0   17.6 29.4   43.75 29.39   38.65 41.21   75.03 76.98   31.97 32.39   A-7-5 A-7-5   31.61 26.15   MH MH   Clayey and Silt Material 54.7   54.7 69.2   15.8 14.3	P-1   P-2   P-3     100   100   100     94.6   95.2   98.6     82.4   70.6   84.2     29.03   34.67   38.57     2.7   2.72   2.74     0   0   0     17.6   29.4   15.8     43.75   29.39   20.1     38.65   41.21   64.1     75.03   76.98   67.88     31.97   32.39   33.19     A.7.5   A.7.5   A.7.5     31.61   26.15   32.74     MH   MH   CH     Clayey and Silt Material   54.7   69.2   58.8     15.8   14.3   14.31   14.31     62.11   67.65   67.44   15.3	P-1   P-2   P-3   P-4     100   100   100   100     94.6   95.2   98.6   92.6     82.4   70.6   84.2   82     29.03   34.67   38.57   31.68     2.7   2.72   2.74   2.75     0   0   0   0     17.6   29.4   15.8   18     43.75   29.39   20.1   35     38.65   41.21   64.1   47     75.03   76.98   67.88   67.41     31.97   32.39   33.19   31.89     4.7-5   A-7.5   A-7.5   A-7.5     31.61   26.15   32.74   30.51     MH   MH   CH   CH     Clayey and Silt Material   58.8   66.3     15.8   14.3   14.31   20.32     62.11   67.65   67.44   70.1



Figure 3. Treatment of soil with varying amount of Lime at 7day curing.

Figure 2 shows the view of conducting a double Hydrometer Test on dispersive soil with a standard hydrometer and parallel hydrometer test.

# 3.2. Chemical analyses of soil

1 g of sample was placed in a 250 ml digestion flask and then heated to 95 °C with 10 ml of 50% HNO<sub>3</sub> without boiling. After cooling, it was refluxed with repeated additions of 65 % HNO<sub>3</sub> until no brown fumes were rising from the sample. After cooling, 10 ml of 30% H<sub>2</sub>O<sub>2</sub> was added tardily, no losses were allowed. The mixture was refluxed with 10 ml of 37% HCl at 95 °C for 15 min. The attained digestate was screened by a 0.45  $\mu$ m membrane filter paper, diluted to 100 ml with deionized water, and kept at 4 °C for assessment. The filtrates were also analyzed for Ca,

Mg, K, and Na by using atomic absorption spectroscopy method. Soil dispersivity is mostly occurred due to the existence of exchangeable sodium present in the structure, which is shown in Table 3 for untreated soil. The erosion due to dispersion of soil depends on mineralogy, clay chemistry, and dissolved salts in pore water.

# 3.3. Classification of subgrade soil

Table 4 explains the basic properties and soil classification of the different pit representative samples summarized for untreated subgrade soil. All representative soil samples gathered from each pit along the road contain more than (67%–76%) of fine-grained soil passing through sieve No. 200 (0.075mm opening) as obtained from wet sieve grain size analysis. The hydrometer analysis was performed to distinguish the





Table 5.	The	influence	of	lime	and	curing	period	on	dispersion.	
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	01 1				
Lime content,%	Pit-2 (MH soil)		Pit-4 (CH soil)		
	7day curing period	14 day curing period	7day curing period	14day curing period	
	Decrease in dispersion (%)		Decrease in dispersion (%)		
0	69.2	69.2	66.3	66.3	
3	55.38	52.6	51.55	50.05	
5	48.73	45.8	45.16	42.98	
7	44.91	39.89	39.2	36	
9	42.6	33.2	35.72	31.09	

Table 6. Variation of Exchangeable cations in treated soil at 7 day curing period.

Lime content	Pit-2	Pit-2							
	Ca2+	Mg2+	Na+	K+	SAR	SP			
0%	0.56	0.31	6.21	2.10	14.30	67.65			
3%	0.98	0.82	6.01	1.91	6.67	61.83			
5%	2.58	2.42	4.59	2.27	1.83	38.70			
7%	2.69	2.55	4.27	2.39	1.63	35.88			
9%	2.81	2.62	4.01	2.58	1.48	33.40			

Table 7. Variation of Exchangeable cations in treated soil at 14 day curing period.

Lime content	pit-2							
	Ca2+	Mg2+	Na+	K+	SAR	SP		
0%	0.56	0.31	6.21	2.10	14.28	67.65		
3%	2.68	1.92	4.58	2.40	1.99	39.55		
5%	2.72	2.51	4.40	2.29	1.68	36.91		
7%	2.83	2.57	4.20	2.42	1.56	34.94		
9%	2.95	2.65	3.89	2.64	1.39	32.07		



Figure 5. Effect of Lime content on Atterberg's limits.



Figure 6. Effect of curing periods on Atterberg's limit.

percentage of silt and clay passing sieve No.200 (0.075mm opening). Therefore, based on AASHTO and USCS soil classification systems, all representative samples fall under two classes of soil material types, which is very high plastic soil of clayey materials and high plastic silty soil under group index of A-7-6 and A-7-5 respectively. Double hydrometer and exchangeable sodium ion play an essential role in soil identification and classification. These parameters are indicators of some geotechnical problems such as dispersivity, excess sodium ion, and sodium absorption. One of the fundamental aims of this study is to evaluate the changes in dispersion and exchangeable cation with the addition of lime.











Figure 9. Effect of curing period on the grain size distribution of subgrade soil.

# Table 8. Classification of lime treated dispersive Soil.

Pit -2	Specific	Grain Size Di	stribution			Atterberg's Limi	t	
	Gravity	Gravel	Sand	Silt	Clay	Liquid Limit	Plastic Limit	Plastic Index
		(%)	(%)	(%)	(%)	LL (%)	PL (%)	PI (%)
0% Lime	2.72	0	29.4	29.39	41.21	76.98	44.59	32.39
3% Lime	2.7	0.46	49.2	21.62	28.72	71.57	46.56	25.01
5% lime	2.65	1.74	51	19.16	28.1	65.41	48.11	17.30
7% Lime	2.63	2.94	52.8	23.36	20.9	58.24	49.03	9.21
9% Lime	2.6	3.26	55.8	17.30	19.567	54.06	49.98	4.08
Grain Size Dist	ributions	Classificati	on			Materi	ials type	Subgrade rating
No. 200 pass		According	to AASHTO	Acc	ording to USCS			
70.6		A-7-5		MH		Mostly	/ Clay soils	Fair to poor
50.34		A-7-5		MH		Mostly	v Clay soils	Fair to poor
47.26		A-7-5		GC		Mostly	v Clay soils	Fair to poor
44.26		A-5		GM		Mostly	v Silty soils	Fair to poor
40.94		A-5		GM		Mostly	v Silty Soil	Fair to poor

### Table 9. Classification of lime treated dispersive soil for 7 and 14 days curing period.

Curing	Lime	Sieve Analysis	Atterberg Limit			Classification	Classification		
Period	Content	Sieve No. 200 Pass (%)	Liquid Limit (LL) %	Plastic Limit (PL)%	Plasticity Index (PI)%	6 According to A	ASHTO Group Index	According to USCS	
7Days	0%	70.60	76.98	44.59	32.39	A-7-5	26.15	MH	
	3%	50.34	71.57	46.56	25.01	A-7-5	10.79	MH	
	5%	47.26	65.41	48.11	17.30	A-7-5	6.36	GC	
	7%	44.26	58.24	49.03	9.21	A-5	2.47	GM	
	9%	40.94	54.06	49.98	4.08	A-5	0.00	GM	
14 Days	0%	70.60	76.97	44.59	32.39	A-7-5	26.15	MH	
	3%	46.70	68.49	45.84	22.66	A-7-5	8.02	GC	
	5%	41.61	60.50	47.59	12.91	A-7-5	2.77	GC	
	7%	34.30	54.12	49.88	4.24	A-2-5	0.00	GM	
	9%	27.10	52.07	50.85	1.22	A-2-5	0.00	GM	
Maximum Density (g	Dry (/cm <sup>3</sup> )	Optimum Moisture Content (%)	CBR with 4-day Soaking	unconfined Co Strength (Kpa	ompression Di )	spersion%	Sodium Absorption Ratio (SAR)	Sodium Percentage (PS)	
1.66		23.12	3.00	99.51	69	.2	14.30	67.65	
1.60		24.66	3.70	131.64	55	.38	6.67	61.83	
1.58		25.06	4.50	137.97	48	.73	1.83	38.70	
1.49		27.33	5.20	176.20	44	.91	1.71	36.90	
1.425		29.43	7.50	201.66	42	.60	1.48	33.40	
1.662		22.8	4.60	99.51	69	.20	14.28	67.65	
1.58		25.00	6.20	146.71	52	.60	6.98	56.79	
1.555		25.50	8.70	165.62	45	.80	1.68	36.91	
1.532		28.00	13.50	189.11	39	.89	1.56	34.94	
1.50		30.23	17.10	222.02	33	.20	1.39	32.07	



# Figure 10. The unconfined compression strength of dispersive soil with lime.

# 3.4. Effect of lime on dispersivity of dispersive sub-grade soil

The soil dispersivity was studied by performing double hydrometer tests, and dispersivity of soil continuously decreased with increasing additions of lime from 69.2% to 42.6% at a 7day curing period. Figure 3 shows a decrease in dispersivity with the addition of lime in soil samples.

# 3.5. Effect of curing periods on lime treated soil on dispersion

When the soil was treated with lime, an increased curing period produced a stronger mixture. Though, the quantity of decrease in dispersivity is variable. The study was undertaken to obtain the effect of various lime percentages (3%, 5%, 7%, and 9%) on a decrease in dispersivity. Subsequently, it was cured for 5, 7, and 14 days to assess the Table 10. Unconfined Compressive Strength at different lime content and curing period.

MH soil	Curing Periods	5 Days	7 days	14 Days
	0%	97.02	99.51	99.87
	3%	100.29	113.61	139.14
	5%	126.11	131.64	146.61
	7%	135.21	137.97	171.67
	9%	161.66	194.67	237.62



Figure 11. Variation in CBR results of penetration Vs. Load after 7 days at different lime percentage content.

effect of long-term curing on dispersion by double hydrometer test and on the lime stabilization process. The evaluation of dispersion soil-lime is shown in Table 5. Figure 4 shows a decrease in dispersivity with increasing lime amount and curing period.

# 3.6. Effect of lime on chemical properties

Tables 6 and 7 show the change of free cations for soil lime combination at different percentages with the curing period. In the specimen, sodium ion concentration was substituted by calcium ions supplied by the contribution of lime. And thus, the values of SAR and PS were less and classified as nondispersive, with all exchangeable cation units in meq/L of saturation extract.

# 3.7. Effects of lime on Atterberg's limits

The effect of lime on soil plasticity was also investigated by performing Atterberg's limits tests. Figure 5 displays that the liquid limit and plasticity decrease while a slight increase in the plastic limit for lime –treated soil is noticed with increment in lime content from 3% to 9%.

As the amount of lime is increased, there is a decrease in clay content and thereby a corresponding increase in the number of coarse particles. These outcomes in a reduction of Plasticity Index at a lime content of 7%, a maximum reduction in the Plasticity Index to 8.22% was obtained. When the lime content was increased beyond 7%, no further changes in the Plasticity Index were observed, even for 7 days curing periods. The results shown in Figure 6 for the soil samples show that the addition of lime to the natural samples decreased their liquid limit, increased their plastic limit, and consequently reduced their plasticity index. The study shows the plasticity index of soil lime treated sample at 7% of lime

Table 11 Variations of CBR result at different lime content and curing periods

content for curing periods of the first 14 days is changed to non-plastic behavior.

From the findings of laboratory results, it can be concluded that 7% of lime content is the optimum dosage to reduce the plasticity index of dispersive subgrade significantly.

# 3.8. Effect of lime on compaction of subgrade soils

Increasing of lime to dispersive subgrade materials (MH soil) increases their optimum moisture content from 22.80% to 30.23% and reduces their maximum dry density from 1.66g/cc to 1.50g/cc for the same compactive effort at 14 days curing period (Figure 7) and CH soil, optimum moisture content increase from 14.2% to 21.20% at 9% lime content and its maximum dry density decrease from 1.52g/cc to 1.46g/cc (Figure 8).

# 3.9. Effect of lime on grain size distributions of subgrade materials

Based on laboratory results mentioned in Figure 9 and Table 8 below, all the samples (untreated and treated with lime) were classified by the Unified Soil Classification and AASHTO classification system by conducting wet sieving tests and hydrometer analysis by following ASTM D 422-63 standard. Figure 9 shows particle size distribution curves of soil and lime mixtures with 7days curing period. Results illustrate the decrease in the fraction of clay and the increase in the sand and gravel fractions due to flocculation reactions shown in Table 8.

The variation of particle size distribution curves occurs for all stabilizers. In general, the soil-lime mixtures demonstrated a decrease in the clay fraction. Calcium exchangeable concentration from hydrated lime facilitated the flocculation reaction. Table 8 shows the clay fractions reduced from 41.21% to 19.57%, Sand fraction increase from 29.4% -55.8%, Gravel fraction increase from 0% to 3.26 at 7 day curing period of subgrade CH soil after addition of 3%, 5%, 7% and 9% of lime.

Table 9 shows that soil classification of treated soil samples at 9% of lime was nonplastic and classified as A-5 according to the AASHTO classification system. This finding indicates that from 7% to 9% lime dosage is optimum for this subgrade to change soil particles from A-7-5 to A-5 and A-2-4. Additionally, the subgrade quality according to AASHTO classification changes from (Fair to sparse) to (Excellent to good).

# 3.10. Effect of lime on unconfined compression strength of subgrade materials

The unconfined compressive strengths of natural subgrade soil performed and varied from 55kPa to 100 kPa, which indicates medium

MH soil Lime Content	7 Days Curing		14 days curing					
	CBR (%)	Swelling (%)	CBR (%)	Swelling (%)				
0%	3.0	14.33	3.0	14.33				
3%	3.7	11.14	5.61	7.66				
5%	4.5	8.73	8.33	5.21				
7%	5.2	6.95	13.79	3.11				
9%	6	4.23	22.31	0.98				

rating subgrade quality. Lime-soil mixture strengths varied widely, based on the soil, lime percentage, and curing period. Figure 10 shows Unconfined Compression Strength with lime content for 5, 7, and 14 days under soaking condition. A higher percentage of lime is noticed to produce higher strength after the curing period.

Unconfined compressive strengths are interpreted with consistency (quality) of the soils used in pavement applications. It was observed from Table 10 that there is an improvement in the Unconfined Compressive Strength of dispersive soil with the addition of lime.

The findings of this study imply that hydrated lime is a suitable additive to stabilize dispersive soils and gained high strength at 9% lime content.

### 3.11. Effect of lime on CBR of subgrade materials

Figure 11 shows the influence of lime stabilized dispersive clay on CBR. Table 11 shows a CBR value increase from 3% to 22.31% at 14 days curing period with an increment of 86.55%.

The CBR value of soil-lime treated was increased significantly at 9% lime content in 7 days as well as 14 days curing period. The study shows that 7% to 9 % of Lime content for 7 and 14 days curing periods was optimum lime content.

# 4. Conclusion

Dispersive subgrade soils pose severe problems to pavements that are constructed over them. Because these soils are easily erodible and also segregate due to variation in moisture content. Such soils also have low load-bearing capacity during wetting. These soils have been a significant challenge to the road construction sector in specific areas of Ethiopia. The sub-grade soil of the untreated sample was grouped as A-7-5 by the AASHTO and MH, CH as per USCS systems, which are generally regarded as poor subgrade quality. The subgrade soil was changed to GC and GM groups of soil after different amounts of lime treatment for the previous 7 days and 14 days curing periods. The outcome of this study suggests that hydrated lime provides promising results in improving the engineering properties of the subgrade soil. The dosage of 7%–9% of the hydrated lime is enough to stabilize the dispersive sub-grade soil pavement of roadbed.

## Declarations

#### Author contribution statement

Bisrat Gissila Gidday and Satyendra Mittal: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

## Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### Competing interest statement

The authors declare no conflict of interest.

#### Additional information

No additional information is available for this paper.

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