STUDY OF DRY MIXING METHOD TO APPLY MICROBIAL-INDUCED CALCITE PRECIPITATION FOR SOIL TREATMENT

by

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DEDICATION

To my beloved parents, who have dedicated their entire lives to ensuring that I receive the finest education and have showered me with boundless love. To my dear brother, for his constant companionship and unwavering presence in my life. To my loving husband, you have consistently been my rock, someone I can always rely on.

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ABSTRACT

Microbially Induced Calcite Precipitation (MICP) has emerged as a promising technique for soil stabilization, traditionally involving the preparation and mixing of treatment solutions with the soil. However, this thesis explores a novel approach of applying MICP by directly mixing dry chemical compounds into the soil and subsequently adding water. This alternative method offers potential advantages in terms of convenience, ease of implementation, and cost savings.

The objective of this research is to investigate the applicability of dry mixing protocols for MICP in soil stabilization. Through comprehensive experimentation, three different dry protocols were developed and applied to five different soils. The effectiveness of these protocols was evaluated by monitoring pH levels, calcium carbonate precipitation, and free swell indexes.

The findings demonstrated that the dry mixing protocols resulted in significant calcium carbonate precipitation, comparable to or even surpassing that of the conventional protocol after some rounds of treatment. This research provides valuable insights into the feasibility and efficacy of employing MICP through dry mixing methods.

The innovative approach of directly mixing dry chemical compounds into the soil and subsequently adding water presents numerous benefits in terms of convenience and cost-effectiveness. By eliminating the need for preparing and mixing treatment

solutions, this approach streamlines the application process, facilitating large-scale implementation.

This research contributes to the advancement of MICP techniques and offers a practical alternative for the soil stabilization industry. The new application method has the potential to revolutionize soil stabilization practices, providing a more efficient and effective solution for various geotechnical applications. Further development and implementation of dry mixing protocols in the industry can lead to significant advancements in soil stabilization practices, ultimately enhancing infrastructure durability. Both the conventional protocol and dry protocol-1 exhibited a similar trend of calcite precipitation as the treatment rounds progressed. In both cases, there was a gradual increase in the amount of calcium carbonate with each successive round of treatment. However, it is important to note that while the conventional protocol resulted in the highest overall calcium carbonate precipitation after seven treatment rounds, dry protocol-2 and dry protocol-3 displayed a distinct pattern. These dry protocols initially generated a relatively substantial amount of calcium carbonate precipitation in the early treatment rounds, but as the rounds progressed, the precipitation either declined or showed minimal increments. This observation underscores the differential behavior of the dry protocols compared to the conventional protocol regarding calcium carbonate precipitation throughout the treatment process.

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CHAPTER 1:

INTRODUCTION

1.1 Background and MICP

The practice of modifying or enhancing soil characteristics to make it more stable and useful for construction is known as soil stabilization. Geotechnical engineers face difficulty providing stable ground for the structures with around 40,000 projects (Cheng and Cord-Ruwisch 2014) worldwide that call for soil repair each year, totaling AUD$6 billion (DeJong et al. 2010). Increased soil durability, decreased erodibility, improved compaction, and increased soil load-bearing capacity are the goals of soil stabilization. The success and durability of any construction project depends heavily on the strength and stability of the soil, making soil stabilization an essential component of civil engineering. In civil engineering, soil stabilization is essential because it ensures that structures constructed on the ground have a strong foundation that can support the weight and load of the structure being built. In addition to decreasing soil erosion, soil stabilization increases the soil's strength, which over time lowers maintenance expenses. Minimizing soil pollution and erosion, which can have detrimental effects on neighboring ecosystems, can also aid in environmental protection.

Chemical stabilization is a widely recognized method employed for enhancing soil stability, which involves the process of enhancing soil properties through the strate-

gic introduction of chemicals. Lime, cement, and fly ash are typical chemical soil stabilizing agents. Stabilization through mechanical techniques, such as compaction or grading, entails changing the characteristics of the soil. This technique is frequently used to increase the soil's ability to support loads. Biological stabilization entails enhancing soil qualities through natural processes and organisms. Utilizing microbial-induced calcite precipitation (MICP), which uses bacteria to produce calcium carbonate minerals in the soil, is one type of biological stabilization. Chemical and mechanical soil improvement methods in the context of geotechnical applications demand a significant energy in terms of material manufacture and on-site operation (Achal and Mukherjee 2015). MICP is an approach that has the potential to alter and enhance the ground condition, which is relatively green, sustainable, and ecologically friendly (Hadi and Saeed 2022). MICP improved soil has an estimated lifespan of 50 years, which is comparable to the anticipated service life of many geotechnical structures (Dejong et al. 2013).

MICP can be applied using either biostimulation or bioaugmentation. Exogenous bacteria are injected into the soil during bioaugmentation to cause calcite to precipitate. According to Burbank et al. (2011), biostimulation uses local bacteria to cause calcite precipitation, and it is quickly gaining popularity as a MICP application technique. This method is advantageous from an economic and environmental standpoint because it does not require costly non-native bacterial cultivation and injection into natural soil ecosystems. These ureolytic microorganisms are tougher than the microbes that are injected, leading to a consistent distribution of calcite and continued enzymatic activity (Gomez et al. 2018). Even with an in-depth knowledge of MICP and a successful field experiment, the bioaugmentation treatment technique

cannot be regarded as a cost-effective alternative due to the infection and cultivation of bacterial strains. Even considering the environmental factor the addition of the bacterial strain can upset the natural eco-system (presence of native bacteria), exogenous bacteria's ability to survive, uneven distribution, the length of time required for bacteria to permeate materials, the cost of cultivation, and the need for extra care when mixing. Therefore, putting the technique into practice on a wide scale presents enormous difficulties. The technique of biostimulation, on the other hand, entails altering environmental factors including substrates, nutrients, and electron acceptors to enhance native microorganisms with desired metabolic skills (Snoeyenbos-West et al. 2000).

1.2 Problem Statement

The acceptance and implementation of Microbially Induced Calcium Carbonate Precipitation (MICP) as a viable substitute for conventional soil enhancement methods in the geotechnical engineering and construction domains are contingent upon the successful outcomes achieved in practical field trials. It is crucial to optimize the treatment methodology to ensure compliance with performance requirements and mitigate the financial costs associated with MICP deployment. Furthermore, there are multiple facets related to MICP application in the field that necessitate thorough examination and analysis. Conducting trials is indispensable for the advancement of effective treatment delivery systems, tailored to various soil stabilization objectives, ultimately facilitating cost reduction during the application process (Ghasemi and Montoya 2022).

Different techniques have been used to apply MICP via biostimulation to soil. The traditional procedure involves preparing enrichment treatment solution by combining

chemical compounds with deionized water. Soil is mixed with this solution. Following the enrichment phase, the soil is repeatedly mixed with a cementation solution, which is a combination of chemical compounds comprising calcium chloride and deionized water.

Dejong et al. (2014) devised a three-dimensional treatment approach for the practical implementation of MICP on a field scale. This method employs a repetitive five-spot pattern consisting of injection/extraction wells to treat an experimental layout measuring 3m by 3m by 0.15m. Each spot pattern involves the placement of one injection well at the center and one production well at each corner of the designated treatment zone.

The mixing of treatment solutions with soil has been researched using a variety of techniques. A treatment solutions delivery system was built with four chambers, each with two sources for enrichment and cementation solutions connected individually. A specific flow pressure could be provided by solutions (Chittoori et al. 2018). Similarly, in another study, eight soil columns were created in autoclaved Teflon cylinders with top and bottom caps (Gomez et al. 2018), allowing treatment solution to be applied from the bottom up. While the techniques discussed above were able to achieve MICP, it is important to note that its applicability at a larger scale may incur substantial costs, and there is a possibility of potential clogging at the injection point.

In a comparative study conducted by Ghasemi and Montoya (2022), three distinct application systems were evaluated: surface spraying, prefabricated vertical drains (PVDs), and shallow trenches. The application process involved the utilization of multiple 1,041-L tanks and pumps for solution administration. For surface spraying, an irrigation setup was employed, consisting of 25 spray nozzles connected

to a primary hose. A pump connected to the tanks supplied the solution to the hose and sprayers. Alternatively, PVDs, also known as wick drains, were employed as a means of delivering the MICP solution. The solution would flow through the channels and infiltrate the soil by passing through a nonwoven geotextile fabric in contact with the surrounding soil. Moreover, 16 shallow trenches, each covered with gravel to ensure uniform distribution of the solution, were used as another treatment method. During treatment, the solution was pumped from the top to fill the trenches. The results indicated that MICP exhibited effective soil improvement capabilities and demonstrated compatibility with all three application methods. However, the surface spraying method only yielded a shallow treated zone. In contrast, the PVD method facilitated the development of a deeper treated zone with a smaller radius of influence. The trench method, while resulting in the highest improvement at the surface, was more localized in its impact.

Hence, while the studies were successful for MICP, they encountered challenges such as the inconvenience and high costs associated with the preparation, supply, and mixing of treatment solutions into the soil. This presents an opportunity for further investigation aimed at discovering novel protocols for MICP that are more convenient and cost-effective. In an endeavor to address this issue, the present research was initiated with the objective of developing new application protocols for MICP. This involved incorporating the necessary chemical compounds in dry form directly into the soil, thus circumventing the need for elaborate preparation of treatment solutions.

Through three dry mixing methods, the biostimulation approach has been employed in this research work. To establish an alternate treatment protocol for stabilizing soils with MICP, this research study is a first step. Consequently, a MICP

strategy has been examined by investigating five soils in this study. To stimulate the bacteria and allow moisture to escape from the mixture, dry enrichment chemicals were mixed with the soil. This was followed by the addition of deionized water. After that, calcium chloride-containing dry cementation compounds were applied, followed by the addition of deionized water for seven rounds for calcite precipitation. For each round, soil samples were cured for 48 hours.

1.3 Research Objective and Tasks

The main objective of this thesis is to develop a novel method for applying microbial-induced calcium carbonate precipitation (MICP) without the need for preparing specialized treatment solutions. This study attempts to enhance and adapt MICP treatment for different applications, making it more practical, simple to use, and potentially less expensive. By examining the effectiveness and viability of the suggested dry technique, which might be flexibly applied to many fields outside of construction, this study seeks to develop a technique that could potentially be both cost-effective and convenient to implement.

The primary objective of this thesis is to explore the applicability of three different Microbial-Induced Calcium Carbonate Precipitation (MICP) techniques for soil stabilization which involves the dry mixing of chemical compounds into the soil, followed by the subsequent introduction of water to achieve the desired calcium carbonate precipitation. The following research objectives were taken into consideration.

1. To formulate and establish protocols for implementing Microbial-Induced Calcium Carbonate Precipitation (MICP) through the dry mixing of chemical compounds with the soil

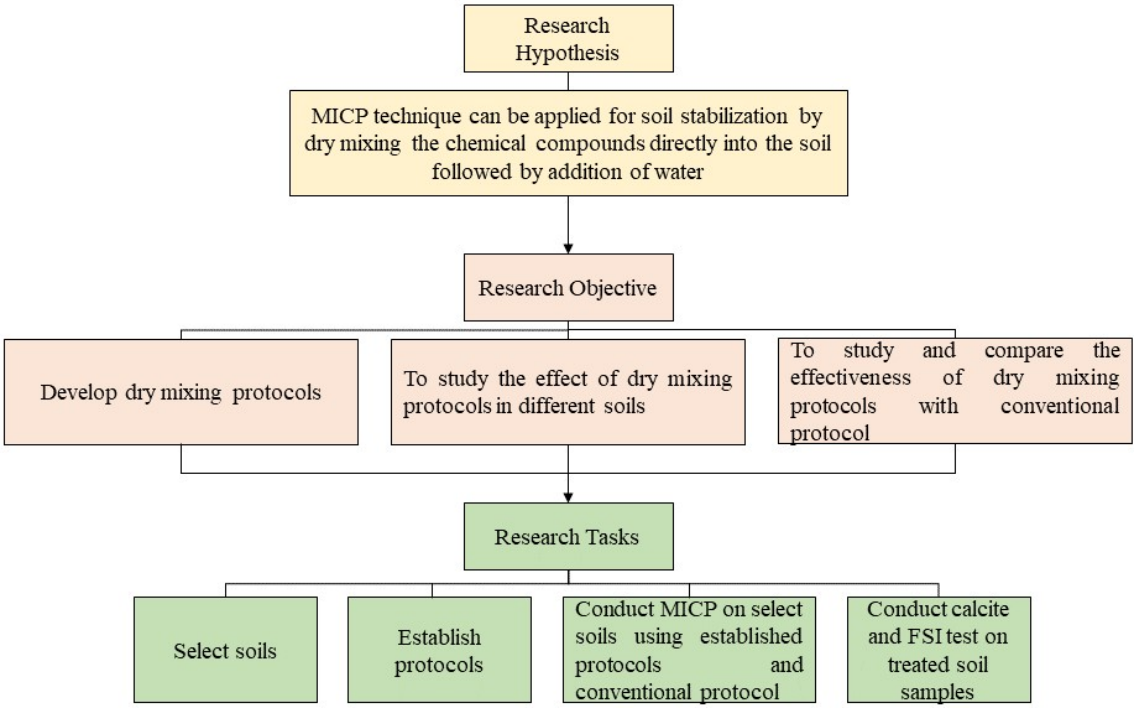

Figure 1.1: Pictorial Representation of Research

2. To examine how different soils respond to dry mixing techniques

3. To look into the impact of dry mixing procedures on the characteristic of soil swelling

4. Investigating how dry mixing techniques affect calcite precipitation

The research tasks to accomplish these research objectives are listed below:

1. Establishing types of soils – To investigate the impact of biostimulation on soils using dry mixing methods, five soils were chosen. Tests such as pH, calcite, and swell tests were used to produce baseline data.

2. Establishing protocols – To treat all five soils, three protocols for dry mixing were studied. The soils were treated with conventional method of MICP as well

and the results were compared.

3. Conduct MICP using conventional and established protocols – MICP was conducted on soils using the conventional and three established protocols. For the established protocols, after treating the soils with dry enrichment compounds and water, dry cementation compounds were added followed by the addition of deionized water.

4. Conduct calcite tests on treated samples – To understand the impact of the established methodology on soils, calcium carbonate determination tests were carried out on untreated and biostimulated soils.

5. Conduct free swell tests on the plastic soils – To evaluate the impact of established protocols on the swelling characteristics of the soil, the Free Swell Index test was performed on untreated and treated soils used in the research.

1.4 Organization of Thesis

Chapter 1 of this master's thesis, which introduces the entire work, is one of five chapters that make up the final product. The review of published literature on the various MICP application approaches that have been researched and published is illustrated in Chapter 2. Chapter 3 describes the conventional method for applying MICP to soils as well as the three established methods. This chapter discusses the procedures followed for each established protocol as well as the tests carried out to determine the protocols' efficacy. The findings from tests carried out to ascertain each protocol's efficacy and to compare them are presented in Chapter 4. It includes graphical representations of data. Here, each plot is explained, and its effectiveness

is discussed. Chapter 5 includes a comprehensive statistical analysis of data generated from our experiment. Chapter 6 provides a summary, a conclusion, as well as recommendations for further study.

CHAPTER 2:

LITERATURE REVIEW

2.1 MICP - Background

Soil holds a crucial position in contemporary construction, serving as the base for various civil infrastructure systems such as buildings, bridges, roads, and dams. Nevertheless, the inherent mechanical characteristics of soils often fall short of meeting the requirements for civil engineering applications. Consequently, soil stabilization techniques are frequently employed in geotechnical engineering to enhance the strength properties of soils (DeJong et al. 2010). In the field of geotechnical engineering, ground improvement is commonly achieved through two predominant methods: mechanical compaction and chemical grouting. However, these methods are associated with several drawbacks, including high costs, substantial energy consumption, and the potential for environmental pollution. To address these limitations, a novel ground treatment technique known as microbial induced carbonate precipitation (MICP) has emerged. This innovative approach, developed relatively recently, offers promising prospects for ground improvement in a more sustainable and environmentally friendly manner (Wang et al. 2017).

Microbial Induced Calcite Precipitation (MICP) is a process that harnesses the ability of urease-producing bacteria to facilitate the formation of insoluble calcite in

the presence of urea and calcium chloride. In the context of soil treatment, MICP can be applied through two distinct approaches: bioaugmentation and biostimulation. Bioaugmentation involves the intentional injection of urease-producing bacteria into the soil, while biostimulation leverages the existing indigenous bacteria within the soil to stimulate calcite precipitation. Previous research has demonstrated that biostimulation holds a competitive advantage as the indigenous bacteria are already acclimated to the soil environment, rendering it a more favorable and effective approach compared to the introduction of augmented bacteria (Islam et al. 2020).

In the biostimulation approach, the indigenous bacteria within the soil are stimulated through the provision of nutrient and carbon sources, resulting in their proliferation and subsequent precipitation of calcite (Burbank et al. 2011). In the context of bioaugmentation, exogenous bacteria are introduced into the soil system. However, the successful establishment and effectiveness of augmented cultures in a new environment are challenging due to the presence of native microorganisms, which can impact their survival rate and metabolic capabilities (Dhami et al. 2017). Numerous research studies have employed the injection of solutions containing a representative ureolytic bacterium, Sporosarcina pasteurii, into soil, followed by the introduction of nutrient solutions to induce calcite precipitation. However, challenges associated with bioaugmentation have been observed, including uneven distribution of bacteria within the soil and clogging near the injection point due to calcite precipitation (Stocks-Fischer et al. 1999). Furthermore, it has been observed that the viability and propagation of exogenous microorganisms introduced into a new environment tend to diminish rapidly. Therefore, in natural environments, such as soils, the efficacy of bioaugmentation with exogenous bacteria is limited due to their reduced compatibility

with the environment and susceptibility to competition and predation by indigenous bacteria (Van Veen et al. 1997). Moreover, the production and transportation of large quantities of these cultures present significant cost and logistical challenges. The injection and uniform distribution of these cultures throughout the treatment site can be technically complex and may face regulatory obstacles (Raveh-Amit and Tsesarsky 2020). The capacity to hydrolyze urea is commonly found among indigenous bacterial populations in soil (Antil et al. 1992). Therefore, the biostimulation approach for Microbially Induced Calcite Precipitation (MICP) in soils is feasible and practical (Raveh-Amit and Tsesarsky 2020).

2.2 Current Challenges of MICP

In the post-COVID-19 era, there is a growing emphasis on reassessing the safety and reliability of biotechnologies. Therefore, it is imperative to exercise caution and thoroughly evaluate the ecological impact caused by the Microbially Induced Calcium Carbonate Precipitation (MICP) process. While some studies have begun investigating the microbial dynamics during MICP implementation, there is still a lack of comprehensive life-cycle analysis from an ecological perspective.

While current research on Microbially Induced Calcium Carbonate Precipitation (MICP) acknowledges the importance of durability, particularly in relation to factors such as freeze-thaw cycles, there is a lack of focus on the potential long-term deterioration of MICP-treated materials. It is crucial to thoroughly assess the degradation of engineering performance over extended periods and under harsh environmental conditions. This evaluation is essential in determining the feasibility of MICP for future applications. Achieving uniform treatment effects remains a significant challenge in the practical application of Microbially Induced Calcium Carbonate Precipitation

(MICP), particularly in large-scale or field-scale scenarios. The proposed approaches to solve this problem are still in the experimental or preliminary stages and require further investigation for practical implementation. Continued efforts are required to reduce the cost and energy consumption associated with Microbially Induced Calcium Carbonate Precipitation (MICP) technology, particularly in the context of the capital-intensive construction industry. It is crucial to explore alternatives to decrease the cost of MICP. These measures would contribute to making MICP a more economically viable and sustainable solution.

MICP emerges as a prominent engineering technology for the future, offering notable advantages in terms of carbon footprint reduction, versatile functionality, and overall convenience. However, the widespread adoption of MICP entails a careful assessment of its implications on ecological balance, environmental impact, and industrial feasibility. Balancing the opportunities and challenges associated with this technology requires prioritizing considerations related to ecological sustainability, environmental responsibility, and practical applicability in various industries (Zhang et al. 2023).

Passive precipitation is the most commonly utilized method of Microbially Induced Calcium Carbonate Precipitation (MICP). MICP has been proposed for various geotechnical engineering applications, including soil strengthening, soil liquefaction mitigation, and foundation settlement reduction. However, the successful implementation of MICP requires determining the most effective chemical treatment and optimizing the process for different conditions. Several factors must be considered, such as the concentrations of chemical reactants and the methods of introducing chemicals into the reaction medium. Clogging near nutrient injection points is a significant chal-

lenge that needs to be addressed, especially at low injection rates. Previous studies have shown that clogging can be prevented with low injection rates and by ensuring a homogeneous distribution of bacterial activity and calcite precipitation. The accumulation of calcium carbonate precipitation is influenced by the injection of chemicals, and the optimal condition occurs when all chemical reactants precipitate as calcium carbonate. A study by Al Qabany et al. (2012) focuses on identifying a chemical delivery technique that maximizes chemical efficiency. Laboratory tests were conducted to examine the impact of factors like chemical concentrations and retention times on chemical efficiency. Understanding the limitations of bacterial activity and reaction rates enables better control of MICP in geotechnical engineering applications. It is crucial to consider how different treatment methods may affect specific applications to ensure optimal results. While the study did not quantitatively measure engineering properties after treatment, it emphasizes the importance of selecting a treatment method that suits field conditions, is optimized for the intended application, and maximizes the efficacy of the MICP process. Therefore, discussion on some of the methods that have been researched and used for application of MICP is as follows.

2.3 MICP – Application Methods

2.3.1 Treatment Solution Delivery System (TSDS)

To conduct the experiments, four chambers were constructed, each consisting of separate sources for enrichment solution and cementation solution. The setup included a PVC chamber with a soil sample placed on a PVC base pedestal, surrounded by latex membranes for surface erosion protection. The top cap and bottom pedestal were designed with grooves and O-rings to securely hold the latex membrane and prevent

water leakage. The top cap had holes for the flow of water and treatment solution, while the bottom pedestal collected the effluent through a puddle arrangement. Once the soil sample was prepared, the PVC chamber was attached to the base plate using a scheduled PVC clear tube, and the treatment solution was introduced through a pipe arrangement from a pressure-regulated water reservoir located above the base plate. The study concluded that the biostimulation approach for Microbially Induced Calcite Precipitation (MICP) in soils is a viable and practical method. However, the practical implementation of this method for treating large volumes of soil in field conditions can present challenges and may not be economically viable (Chittoori et al. 2018).

2.3.2 Injection Method

In a study in Curtin University, Western Australia, treatment was carried out by injection. Initially, a single injection of the cementation solution was administered into the sand column, followed by a 24-hour reaction period at room temperature. Subsequent injections of the cementation solution were repeated to achieve a thoroughly bio-cemented sample with enhanced strength properties. The study observed the occurrence of injection end blocking, characterized by excessive cementation and minor precipitation of calcium carbonate within the column. This led to challenges in conducting further treatments due to severe clogging. The phenomenon of clogging was likely caused by the significant hydrolysis of urea and subsequent formation of calcium carbonate at the injection end. The presence of urease activity and the duration of exposure of the cementation solution to the clogging area were identified as factors influencing this process. Based on the obtained results, it was concluded that the application of the bio-cementation treatment using the injection method

may not be suitable for soils with a lower clay content. This is due to the immediate bio-clogging that occurs at the injection end, where bacterial cells obstruct the pores (Mujah et al. 2016).

A study conducted at Boise State University investigated the application of in-situ fluid injections to induce bio-stimulated calcite precipitation in expansive soils (Pathak 2020). The research involved inserting a pneumatic packer tube into designated injection points for delivering solutions into the soil. The packer tube's rubber lining was inflated using a hand pump to seal the holes, and injections were performed at pressures ranging from 14 psi to 20 psi. During the injection process, the surrounding soil underwent deformation, creating a gap between the packer lining and the soil, which allowed the treatment solutions to escape upward. Approximately 4 gallons of solution were successfully injected per point during each injection round. Leakage of the solution from one injection point to another was observed. Specifically, the flow of solution occurred between two points during the injection of the enrichment solution and the first round of cementation solution. However, during the second round of cementation injections, the flow of injection was observed between different points. This change in flow path could be attributed to the blockage of flow lines caused by the gradual precipitation of calcite in the soil. It was hypothesized that as calcite precipitation took place and particles bonded together, the initial flow path became restricted. Consequently, subsequent injections at the same point resulted in the solution finding alternate pathways.

A research on stimulation of native microorganisms for biocementation in samples recovered from field-scale treatment depths was carried out (Gomez et al. 2018). The soil columns were treated with solutions using a sterile application system, employ-

ing 50-mm 0.22-µm filters for air-displacement and flame-resistant glass connections for sterile exchange of treatment influent solutions. Autoclaved silicone tubing was utilized to convey the solutions at a consistent flow rate of 25 mL/min, facilitated by two calibrated peristaltic pumps. The influent treatment solutions were contained in 1-L Erlenmeyer flasks, enabling multiple treatments from the same flask, and the injection volumes were determined by measuring changes in flask masses during the pumping process. The findings of this study indicate that the stimulation of indigenous ureolytic microorganisms in natural soil deposits can effectively induce calcite precipitation. However, the application and preparation of the experimental setup used in this study may be costly and impractical for field-scale implementation.

Despite the extensive laboratory-scale studies conducted, significant uncertainties remain regarding the overall efficacy of these techniques when applied on a large scale. The implementation of treatment solutions through injection typically involves the use of intricate injection machinery, which can result in substantial costs, especially when considering large-scale field applications.

2.3.3 Surface Percolation Method

Researchers (Cheng and Cord-Ruwisch 2014) in Australia developed a method for applying Microbially Induced Calcite Precipitation (MICP) to sandy soil using surface percolation. They conducted experiments at a depth of 1 meter to demonstrate the feasibility of in situ soil stabilization under a free-draining environment. In their trials, they observed that repeated treatments of fine sand particles ($<$0.3 mm) resulted in clogging and closure at the injection end, limiting the depth of cementation to less than 1 meter. However, this clogging issue was not observed in columns filled with coarse sand particles ($>$0.5 mm) at a depth of 2 meters. Four vertically positioned

PVC columns were filled with fine or coarse-grained silica sands. The solutions were applied to the top of the sand columns and allowed to percolate through gravity and capillary forces, with excess solution draining from the bottom. The continuous application of the solutions created a 5 cm-high ponding on the top surface of the sand until percolation was complete. The vertical profiles of the cemented fine sand columns showed a decrease in CaCO3 content and strength with depth. The results indicated that the surface percolation technique is particularly effective for porous granular materials with high permeability, such as coarse sand and gravel. These materials allow for unobstructed flow of the MICP solution, enabling consolidation to larger depths (over 2 meters). However, for fine sand particles smaller than 0.3 mm, the slow infiltration rate limited the cementation to a depth of 1 meter.

2.3.4 Spray Method

A study in India (Dagliya et al. 2022) was done to assess the feasibility of employing the microbially induced calcium carbonate precipitation (MICP) by spraying technique for mitigating wind-induced erosion in calcareous desert sand. Calcite content percentage was measured to evaluate the effectiveness of the MICP treatment. The spray method was utilized for MICP treatment at a constant temperature of 36°C to simulate field conditions. The treatment process involved daily preparation of the cementation media solution and spraying it onto the sand samples, which were then maintained in an oven at an average temperature of 36°C. The samples were sprayed at 24-hour intervals. The application of cementation solution through the spraying technique resulted in the formation of a thin, rigid layer on the sand surface, effectively covering the dust and preventing wind erosion. The results demonstrated that the biocemented sand samples exhibited reduced erosion compared to untreated sand.

2.3.5 MICP for Erosion Control

A research by Jiang and Soga (2017) investigated the feasibility of using Microbially Induced Calcite Precipitation (MICP) for controlling internal erosion in gravel-sand mixtures. For this, a large one-dimensional column test apparatus incorporating an MICP implementation unit was developed. The apparatus consists of several components, including a pressurized chamber, an axial loading system, a hydraulic control system, a sanding collection system, an MICP implementation system, and an instrumentation system.

The pressurized chamber comprises a hollow column and aluminum pedestal/top plates. A specially designed double-layer base mesh is installed between the pedestal and the column to provide rigidity while allowing only sand to pass through. The axial loading system consisted of a porous loading plate, a pneumatic cylinder, an air pressure regulator, and an iron reaction frame. The loading plate featured holes for water dissipation, and a three-layer sealing system prevents leakage between the piston rod and the top plate. The hydraulic system included a water pressure regulator to maintain a constant hydraulic pressure, with a top mesh placed between the loading piston and the tested soil to distribute inflow water evenly. The sanding collection system consists of Erlenmeyer flasks to collect the outflow containing fluidized sands from the apparatus. The MICP cementation solution was pumped into the hollow column to saturate the soil.

The calcium carbonate precipitation content in the soils showed a consistent increase with higher cementation concentrations, albeit with non-uniform distribution. Increased calcite precipitation correlates with reduced erosion weight, regardless of the applied hydraulic pressure. The formation of cemented sand particle clusters

played a significant role in mitigating soil erosion. Various instrumentation devices were used in the study, including pressure transducers (PTs), a differential pressure transducer (DPT), and a linear variable displacement transducer (LVDT) which can result in substantial costs, especially when considering large-scale field applications.

CHAPTER 3:

MATERIALS AND METHODS

This chapter is centered on the materials and methods employed to accomplish the research objectives. The study investigated the effects of three dry Microbial Induced Calcite Precipitation (MICP) protocols on various soils by analyzing their calcite precipitation. The research involved subjecting five distinct soils to three different dry protocols and comparing the outcomes of calcite precipitation with those of the conventional MICP method. To gauge the effectiveness of the three dry protocols on soils with clay content, the free swell index was evaluated.

3.1 Materials

3.1.1 Soils

Table 3.1 provides a comprehensive examination of the physical properties of the natural soils utilized in the research. A total of five distinct soils were employed, with four originating from Idaho and one from Florida. Among the Idaho soils, two clays were obtained from different locations in Marsing, Idaho while the third soil consisted of sand and the fourth was a subbase soil. The Florida soil sample was classified as fine sand. The gradation and Atterberg's limits for the soils are shown in Table 3.1.

Table 3.1: Physical Properties of Soils used in Research

Soil	Gradation				USCS Classification	ASTM D4318	
	Gravel(%)	Sand(%)	Silt(%)	Clay(%)		Liquid Limit (%)	Plasticity Index (%)
Marsing Soil-1	0	3	1	96	CH	100	60
Idaho Sand	0	99	1	0	SP	NA	NA
Florida Sand	0	99.75	0.25	0	SP	NA	NA
Marsing Soil-2	0	0	9	91	OH	125	62
Subbase Soil	26.13	70.27	3.6	0	SW	NA	NA

One of the five soils used for protocol testing was a mixture of sand and clay obtained from Idaho. The artificial blend was prepared by combining sand and clay in equal proportions. Soil classification was determined based on the Unified Soil Classification System (USCS), a widely adopted framework in geotechnical engineering. Soil gradation was determined for all soils through sieve and hydrometer analysis, conducted following ASTM D422 standards. Additionally, the clays employed in the research underwent Atterberg limits testing to assess their plasticity characteristics.

The notation utilized to differentiate and identify the various soils in the research findings is further elucidated in Table 3.2.

Table 3.2: Notation of Soils used in this research.

Soil Notation	Soil Type
S1	Florida Sand
S2	Idaho Sand
S3	Subbase Soil
S4	50% Idaho Sand + 50% Marsing Soil-1
S5	Marsing Soil-2

3.1.2 Gradation

Sieve analysis and gradation, performed in accordance with ASTM D422, are integral procedures for assessing soil properties. Sieve analysis entails the meticulous separation and determination of particle size distribution in soil samples. The process commences with the precise measurement of a representative soil sample, subsequently subjected to incremental sieving through a series of standardized sieves with progressively diminishing apertures. Each sieve retains particles falling within a specific size range, enabling the computation of the percentage of soil passing through or retained on each sieve. The resultant dataset serves as the foundation for constructing a comprehensive gradation curve, elucidating the spatial distribution of various particle sizes within the soil specimen. This curve graphically depicts the relative propor-

tions of coarse and fine particles, facilitating soil classification and characterization. Thorough analysis of the gradation curve allows for the determination of the overall gradation characteristics of the soil, providing valuable insights for engineering and geotechnical applications.

3.1.3 Atterberg Limit Test

Atterberg limit tests are essential for assessing the consistency of soil by determining key properties such as liquid limit (LL) and plastic limit (PL). These parameters are crucial in characterizing the potential swell-shrink behavior of soils, along with their corresponding plasticity indices. The plasticity index (PI), obtained by calculating the difference between LL and PL values, provides insights into the soil's plastic characteristics. In accordance with ASTM D4318, the Atterberg limit test was performed on three of the soils used in this research, specifically those containing clay components. Soil samples, which had undergone oven-drying at 105^{o}C and were sieved through a number 40 sieve, were selected for the test. This standardized method ensures consistent and reliable results. It is important to note that if the PI exceeds 35, it indicates a high potential for soil swelling, as outlined by the Army U.S. guidelines in 1983.

3.2 Treatment Methodologies

This section of the thesis presents the methodologies employed for each treatment approach, as well as the diverse testing procedures utilized to assess performance. In order to evaluate the efficacy of Microbially Induced Calcium Carbonate Precipitation (MICP) through biostimulation using various dry protocols, three distinct dry methods were employed, namely Dry Protocol-1, Dry Protocol-2, and Dry Protocol-3.

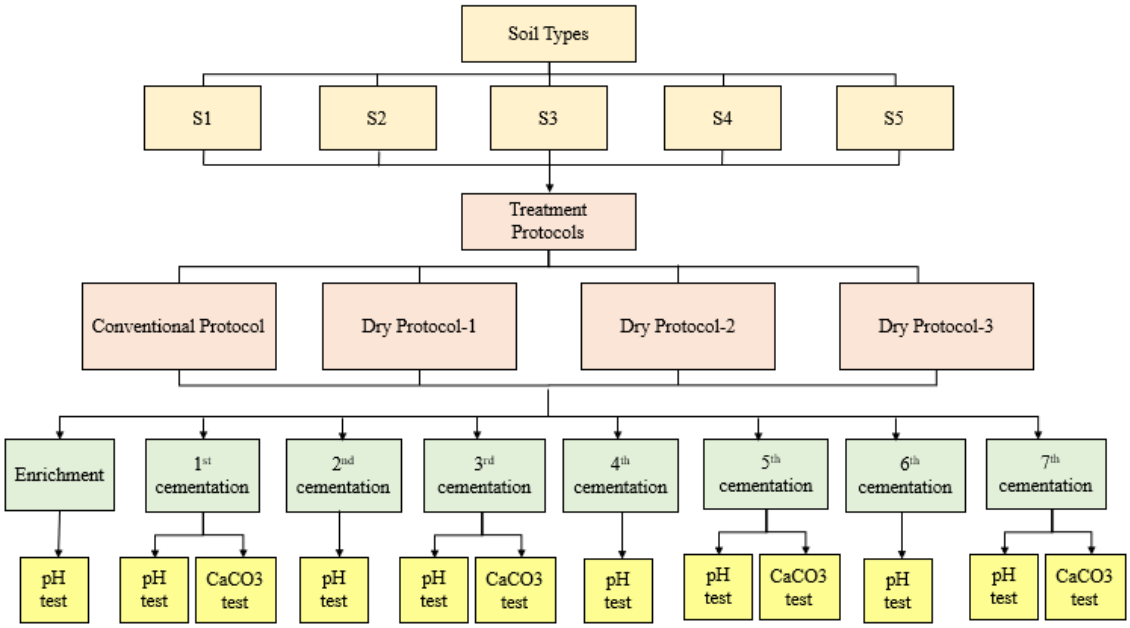

Figure 3.1: Schematic of materials and methods used in this research

Additionally, a conventional MICP method was applied to all soil samples, and the outcomes were compared to those obtained from the three dry protocols to ascertain their effectiveness. To ensure repeatability, two soil samples were taken for each of the four treatment methods, and all soil samples underwent an enrichment process prior to cementation. Prior to initiating any treatment, the pH levels and calcium carbonate precipitation of all soil samples were measured to establish baseline data for each soil type. This preliminary assessment provided crucial control measurements of pH and calcium carbonate content, serving as reference points for subsequent comparative analysis and evaluation throughout the treatment process. A comprehensive flowchart illustrating the complete experimental procedure and testing protocols is presented in Figure 3.1.

3.2.1 Treatment Stages

3.2.1.1 Enrichment Stage

The soil treatment process initiated with a 48-hour enrichment phase, during which the soils underwent a thorough mixing with enrichment compounds in either dry or solution form, based on the specific protocol requirements. Water was added as necessary during the enrichment stage to maintain continuous submersion of the soil, promoting optimal bacterial growth. Following the completion of the enrichment phase, the pH of the solution shifted towards alkalinity for all soil samples, signaling the successful progression into the subsequent cementation stage.

3.2.1.2 Cementation Stage

Following the completion of the 48-hour enrichment stage, the soil samples underwent 7 successive cementation stages, wherein cementation compounds were introduced in either dry or solution form depending on the specific protocol requirements. Water was added in between cementation cycles as required to maintain continuous submergence of the soil samples throughout the process.

During the cementation stages, the pH levels and the formation of calcium carbonate within the soil samples were diligently tested and observed. These measurements served as indicators of the effectiveness and success of the cementation stage, providing valuable insights into the progress and outcomes of the soil treatment process.

Table 3.3 presents a comprehensive overview of the compounds employed during the enrichment and cementation stages.

Table 3.3: Concentration of Chemicals Used for Enrichment and Cementation

Chemicals	Concentration (g/L)	
	Enrichment	Cementation
Urea	20	20
Sodium Acetate Anhydrous	8.2	4.1
Solulys	0.5	0.5
Calcium Chloride	-	27.74

3.2.2 Treatment Protocols

3.2.2.1 Conventional Protocol

Soil samples were collected using moisture cans or porcelain basins as appropriate containers. The initial phase of the treatment involved introducing specially formulated enrichment solutions to the soil samples, ensuring meticulous mixing to achieve a homogeneous mixture. Sufficient volume of the solution was added to completely submerge the soil samples. Following this, the samples were allowed to rest undisturbed at room temperature for a precisely timed period of 48 hours, after which pH levels were measured. It is noteworthy that all samples exhibited an alkaline pH environment (above 7), indicating that the 48-hour duration was optimal for initiating the bio-stimulation process effectively.

After the 48-hour period, each soil sample was carefully drained of any excess solution and combined with the cementation solution, ensuring complete submersion. The samples were then subjected to the controlled and undisturbed cementation process, with pH measurements conducted at regular 48-hour intervals throughout seven consecutive bio-cementation cycles. After each pH measurement, the resulting leachate from the soil samples was diligently drained, and fresh cementation solution was introduced to maintain optimal conditions. The entire cementation process spanned a duration of 14 days, encompassing meticulous assessment of calcium carbonate precipitation after the first, third, fifth, and seventh rounds of cementation treatment.

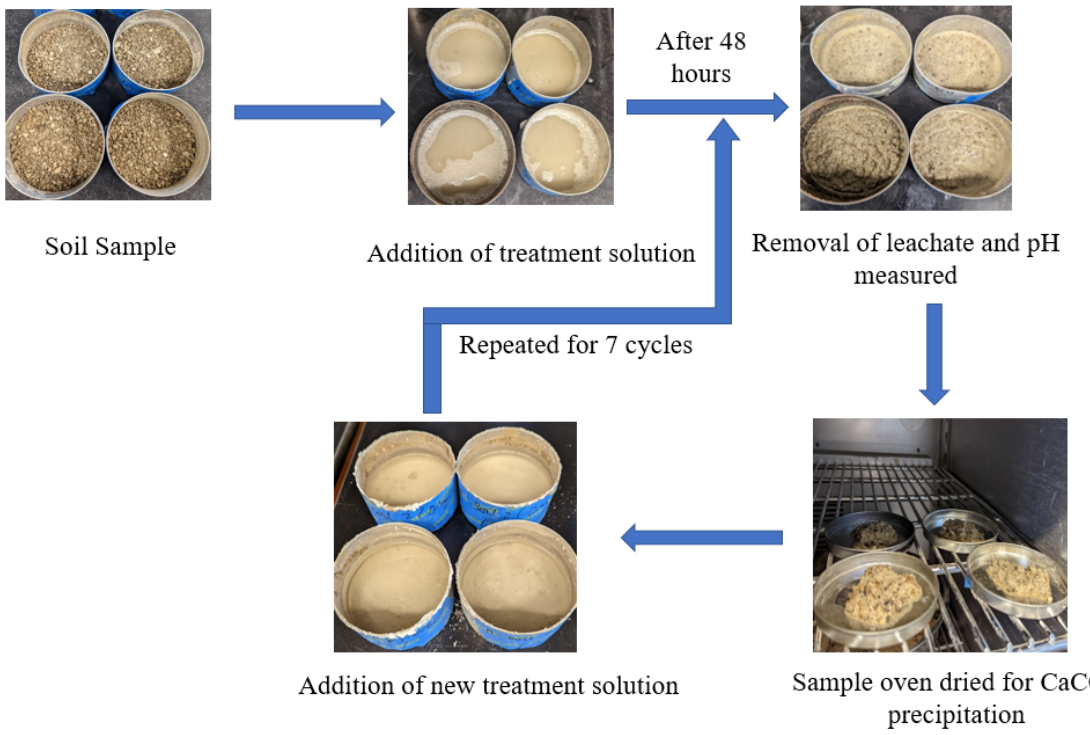

Figure 3.2: Steps involved in treating soil with MICP using Conventional Protocol

3.2.2.2 Dry Protocol-1

Soil samples were collected utilizing moisture cans or porcelain basins as appropriate containers. The treatment procedure commenced by introducing dry enrichment compounds into the soil samples, ensuring thorough mixing for uniform distribution. Subsequently, deionized (DI) water was added in sufficient quantities to fully submerge the soil samples. Following this, the samples were left undisturbed on the countertop for a precisely timed period of 48 hours, after which their pH levels were accurately measured.

After the initial 48-hour period, each soil sample underwent a carefully orchestrated cementation stage. Dry cementation compounds for the first cycle of cementation were added and thoroughly mixed with the drained soil sample, followed by the addition of DI water to ensure complete submersion. It is essential to note that no further mixing occurred after the addition of DI water. Each cementation cycle lasted 48 hours, during which the samples were left undisturbed to allow the cementation process to take place. pH measurements were conducted after every cycle throughout the duration of the seven bio-cementation cycles. After each pH measurement, the leachate resulting from each soil sample was promptly drained, and fresh dry cementation compounds for the next cycle were added and mixed with the soil sample. DI water was subsequently introduced for the subsequent round of treatment, again without any further mixing. The entire duration of the experiment, including stimulation stage encompassed a total of 16 days, with calcium carbonate precipitation measurements performed after the first, third, fifth, and seventh rounds of cementation treatment.

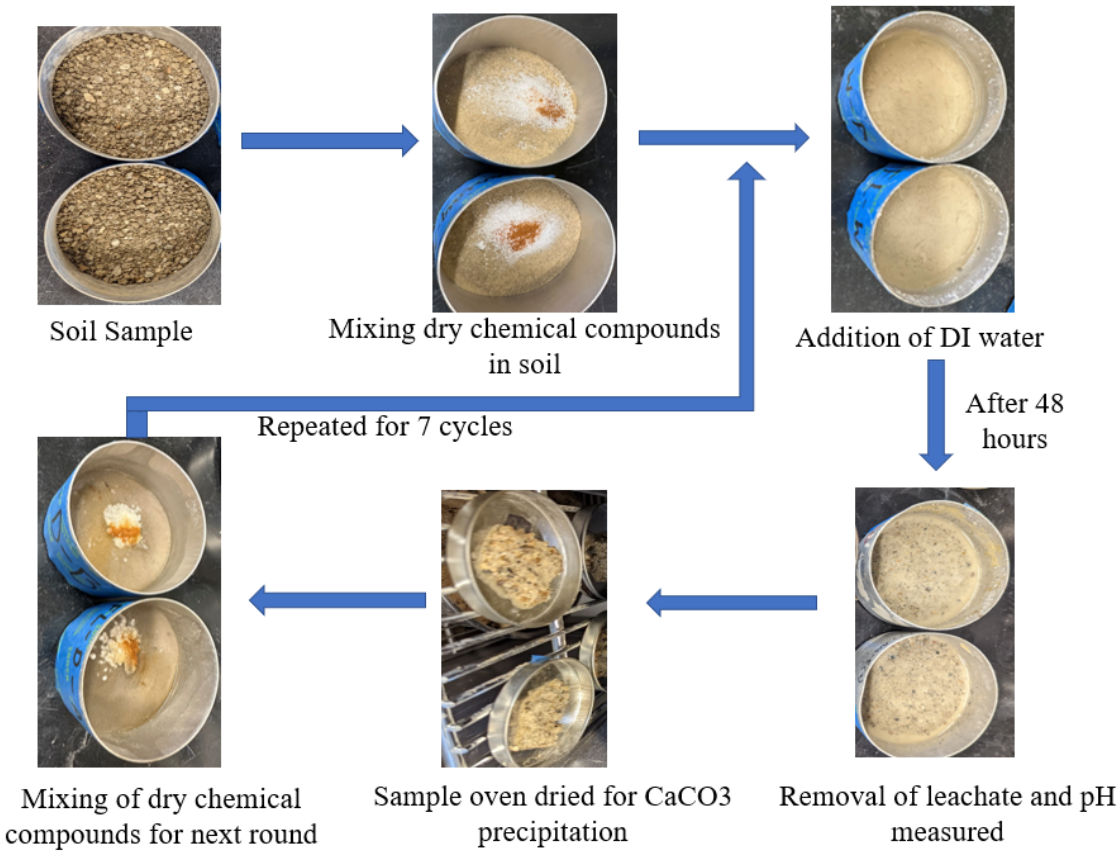

Figure 3.3: Steps involved in treating soil with MICP using Dry Protocol-1

3.2.2.3 Dry Protocol-2

Soil samples were collected in moisture cans or porcelain basins as suitable containers. The treatment procedure commenced by introducing dry enrichment compounds and thoroughly incorporating them into the soil samples. Subsequently, an appropriate volume of deionized (DI) water was added to ensure submergence of the soil samples. After an undisturbed incubation period of 48 hours on the countertop, the pH levels of the samples were measured, exhibiting an increase beyond the neutral value of 7, indicating the readiness to proceed with the bio-cementation process.

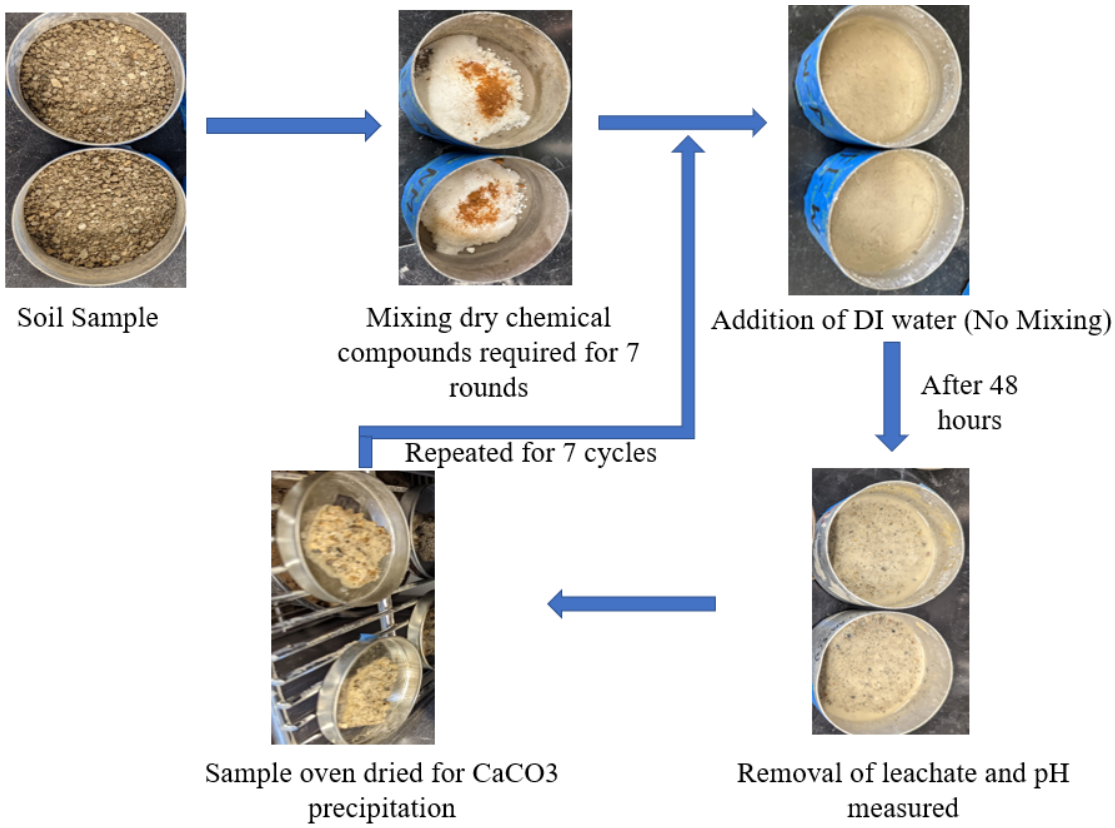

Figure 3.4: Steps involved in treating soil with MICP using Dry Protocol-2

Following the 48-hour stimulation period, each soil sample was drained, and the necessary dry cementation compounds required for the seven planned treatment rounds were meticulously integrated into the soil. Thorough mixing was performed to ensure uniform distribution. Subsequently, DI water was added to achieve submersion of the samples, without engaging in any mixing. The samples were left undisturbed for a period of 48 hours to undergo a single cycle of cementation. The pH levels of the samples were monitored after each cycle throughout the entire duration of the seven bio-cementation cycles. After pH measurement, the leachate resulting from each soil sample was promptly drained, and without causing any disturbance to the

soil samples, fresh DI water was added in preparation for the subsequent treatment round, without any additional mixing. The resulting calcium carbonate precipitation was accurately measured after the completion of the first, third, fifth, and seventh rounds of cementation treatment.

3.2.2.4 Dry Protocol-3

Soil samples were collected in moisture cans or porcelain basins. The treatment process commenced by introducing dry enrichment compounds and thoroughly blending them into the soil samples, ensuring homogeneous distribution. Subsequently, an appropriate quantity of deionized (DI) water was added and mixed extensively to achieve complete submersion of the soil samples. Following an undisturbed incubation period of 48 hours on the countertop, the pH levels of the samples were measured, indicating an increase beyond the neutral value of 7, signifying the readiness to proceed with the bio-cementation process.

After the 48-hour stimulation period, each soil sample was drained, and the requisite dry cementation compounds required for the seven planned treatment rounds were meticulously incorporated into the soil, ensuring thorough mixing. DI water was then added to ensure complete submersion of the samples. In this case, mixing was performed after the addition of DI water. The samples were left undisturbed for a duration of 48 hours to undergo a single cycle of cementation. The pH levels of the samples were diligently monitored after each cycle throughout the entirety of the seven bio-cementation cycles. Subsequent to pH measurement, the leachate resulting from each soil sample was promptly drained, and an adequate quantity of DI water, sufficient for complete submersion of the soil, was mixed in preparation

for the subsequent treatment round. The resulting calcium carbonate precipitation was measured after the completion of the first, third, fifth, and seventh rounds of cementation treatment.

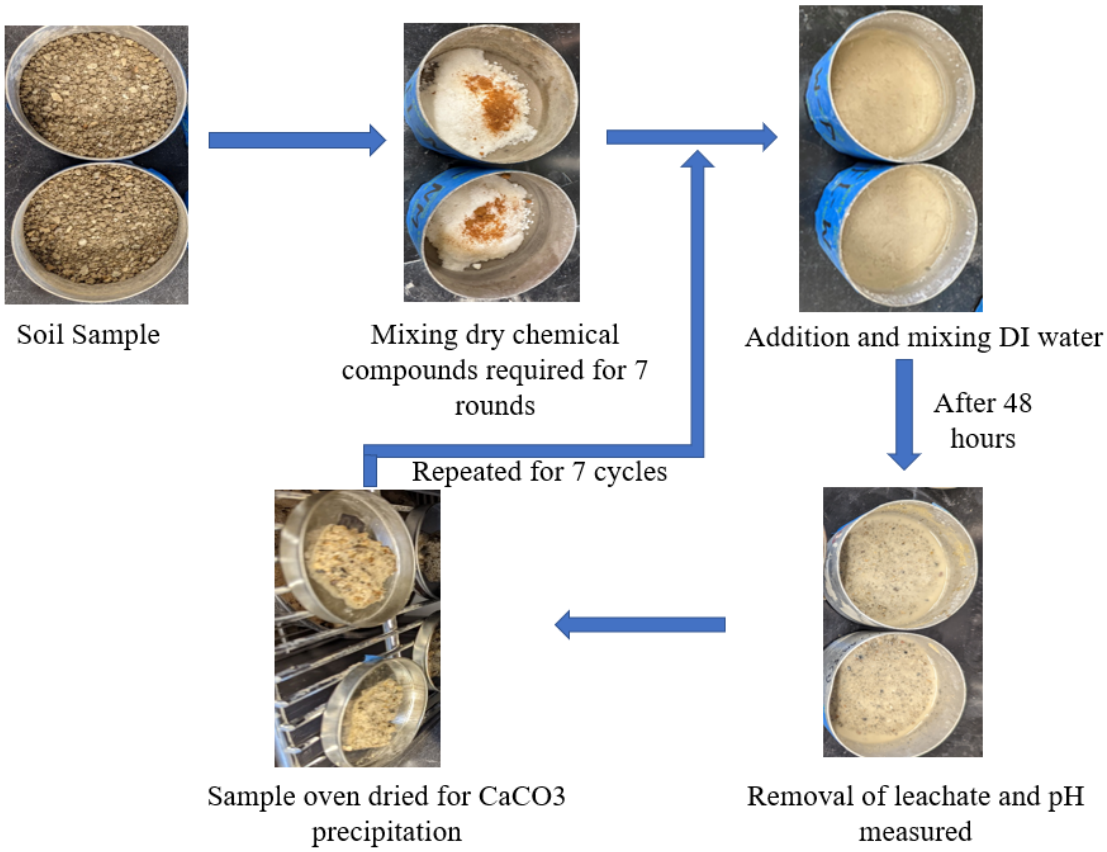

Figure 3.5: Steps involved in treating soil with MICP using Dry Protocol-3

3.2.3 Evaluation Tests

3.2.3.1 pH Test

pH testing was performed utilizing a precise pH meter as shown in Figure 3.6 to assess the acidity or alkalinity of the soil samples. The soil samples were carefully collected

Figure 3.6: pH Meter

and prepared for testing, ensuring representative and homogenous specimens. Prior to testing, the pH meter was calibrated following established protocols to guarantee accurate measurements. Each soil sample was mixed with a suitable volume of distilled water, creating a slurry with a consistent ratio. The pH meter electrode was then immersed into the slurry, and the pH value was recorded. This procedure was repeated for each soil sample to obtain a comprehensive set of pH measurements. The implementation of the pH meter ensured precise and reliable readings, enabling the precise determination of the pH levels in the soil samples.

3.2.3.2 CaCO3 (Calcite) Content Determination Test

The presence of precipitated calcium carbonate was identified utilizing the Rapid Carbonate Analyzer as shown in Figure 3.8. This analytical method involves the reaction between calcium carbonate and hydrochloric acid (HCl), as depicted in the

following chemical equation:

$$CaCO_3 + 2\,H^+ \xrightarrow{\text{Yields}} Ca^{2+} + H_2O + CO_2$$

A dry soil specimen weighing approximately 20g was subjected to sieving using a number 10 sieve, followed by its transfer into a dedicated reactor chamber. Within the reactor, a plastic beaker containing precisely 20±2 mL of hydrochloric acid (HCl) solution with a concentration of 1N was carefully placed. Subsequently, the reactor chamber was hermetically sealed by closing the lid and ensuring proper closure of the pressure relief valve. To facilitate the reaction between the acid and soil sample, the chamber was gently swirled, promoting thorough mixing.

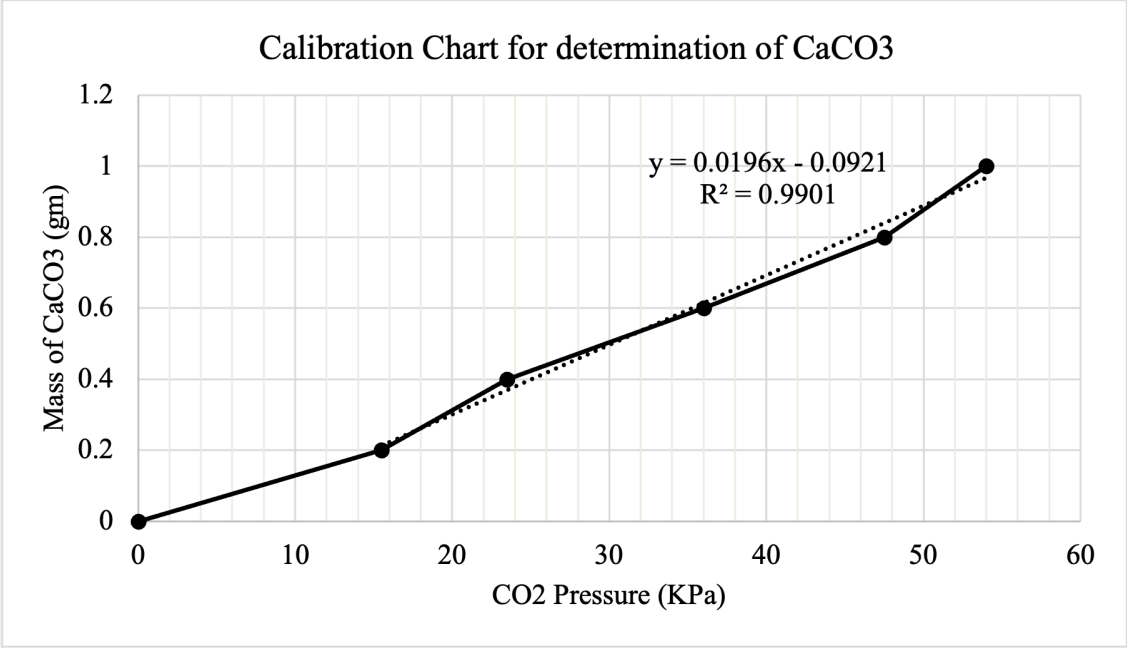

Figure 3.7: Calibration Chart for determination of $CaCO_3$

Following a precisely timed 10-minute interval for the reaction to occur, the pressure within the chamber was measured. The quantification of calcium carbonate ($CaCO_3$) content in the soil sample was determined by referencing a calibration chart

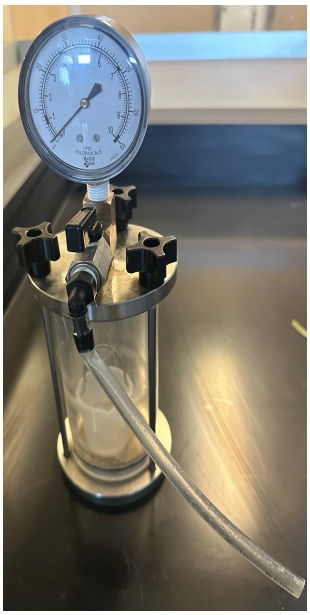

Figure 3.8: Rapid Carbonate Analyzer

as shown in Figure 3.7, which provided accurate readings based on the recorded pressure measurement.

3.2.3.3 Free Swell Index Test

The free swell index serves as a straightforward experimental method employed to assess the potential expansion of a given soil (Holtz and Gibbs 1956). It quantifies the volumetric increase experienced by the soil when submerged in water, without any external constraints. In this test, two samples, each weighing 10 grams and obtained from oven-dried soil samples that had undergone treatment and passed through a number 40 sieve, were carefully poured into separate graduated cylinders with a capacity of 100 ml, employing a funnel for precision. One cylinder was filled with distilled water, while the other was filled with kerosene up to the 100 ml mark. To eliminate entrapped air, gentle shaking and stirring with a glass rod were employed.

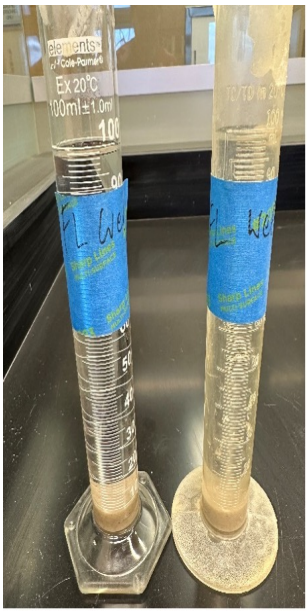

Figure 3.9: Test of Free Soil Index

The soil samples were then allowed to reach a state of equilibrium, where their volumes remained unchanged for a period of 24 hours. Finally, the final volumes of the soil samples in both cylinders were recorded for analysis and comparison as shown in Figure 3.9. The FSI is measured using the equation below:

$$Free\,Swell\,Index\,(FSI)(\%) = \frac{V_d - V_k}{V_k} \times 100 \tag{3.1}$$

where V_d is the volume of the soil sample from the graduated cylinder containing distilled water and V_k is the volume of the soil sample from the graduated cylinder containing kerosene.

CHAPTER 4:

RESULTS AND DISCUSSION

This chapter entails an examination of the outcomes obtained from laboratory tests conducted within the context of this research. For each individual test, a succinct overview of the findings is provided, which is subsequently followed by a comprehensive analysis and examination of these results.

4.1 pH

The pH measurements of the five soil samples, subjected to the four protocols, were documented and presented in Figure 4.1 through Figure 4.5. The observed elevation in soil pH values signifies the creation of a conducive environment for the precipitation of calcium carbonate. Conversely, a decline in pH levels may be attributed to diminished or negligible bacterial activity.

In the case of soil sample S1, as depicted in Figure 4.1, the pH exhibited a notable increase from its initial control value of 7.4 to approximately 9 following the stimulation or enrichment process. This rise in pH indicated the occurrence of urea hydrolysis and subsequent formation of carbonate ions, which are essential for the precipitation of calcium carbonate during subsequent cementation cycles. The pH trends for both the conventional protocol and Dry Protocol-1 displayed similar patterns, with minor fluctuations leading to marginal decreases in pH throughout the

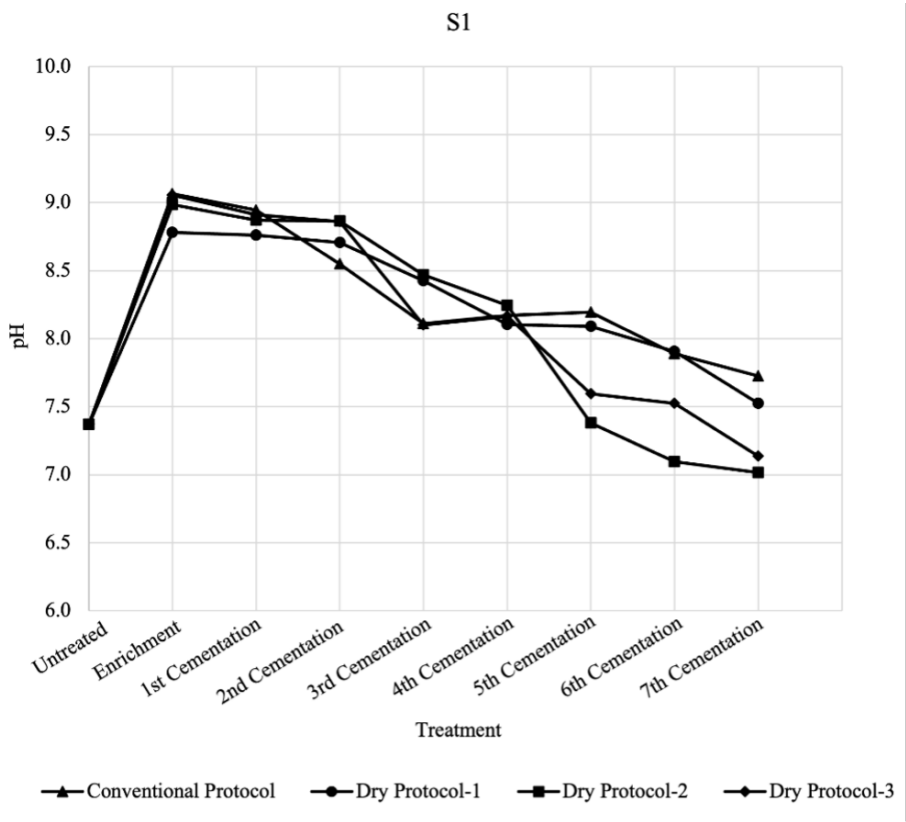

Figure 4.1: Change in pH of soil S1 with treatment rounds.

seven rounds of cementation. Conversely, dry protocol-2 and dry protocol-3 demonstrated a more pronounced gradient in pH between the fourth and fifth cementation rounds. In these cases, the pH experienced a drop from around 9 after the stimulation round to approximately 7 after the end of treatments.

In relation to soil sample S2, as illustrated in Figure 4.2, the pH levels underwent an increase from the initial control value of 7.9 to 8.8 subsequent to the stimulation treatment across all four protocols. Throughout the cementation treatments, the pH remained relatively stable for the conventional protocol, with only a slight decline to 8 by the conclusion of the seventh round. Notably, the pH values associated with dry

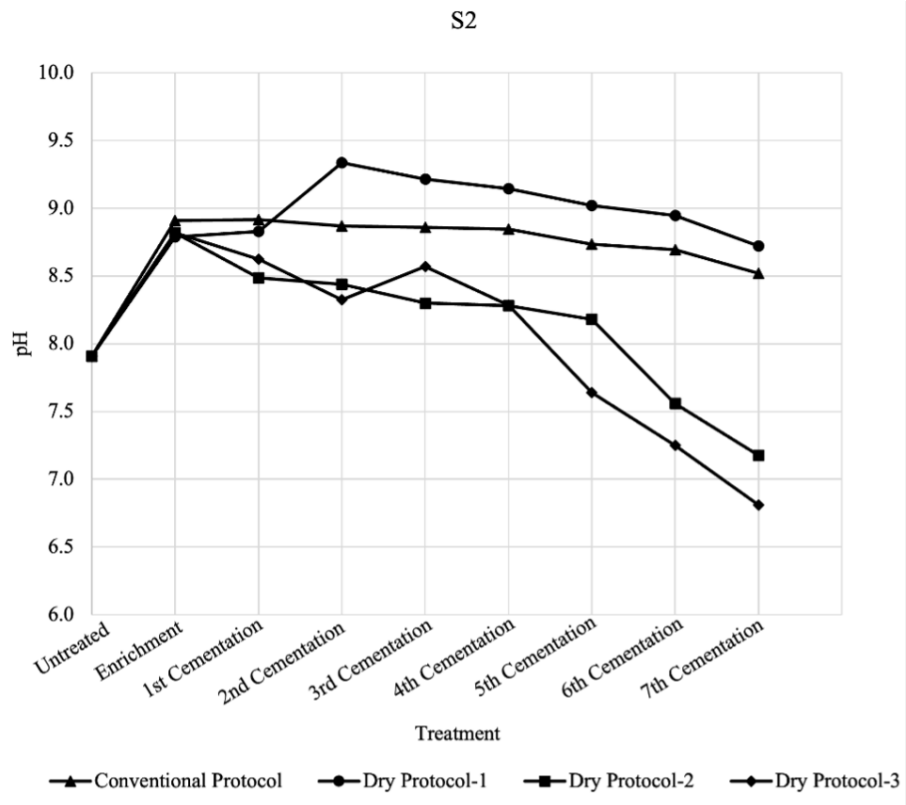

Figure 4.2: Change in pH of soil S2 with treatment rounds.

protocol-1 exhibited the highest levels throughout all treatment rounds, starting at 8.9 and reaching a peak of 9.3 after the second cementation round. Subsequently, there was a gradual decrease, and at the culmination of the seven cementation rounds, the pH recorded was 8.7. For dry protocol-2 and dry protocol-3, the pH patterns observed for soil sample S2 displayed considerable similarities. Notably, a sharp decline in pH was evident after the fourth and fifth cementation rounds for dry protocol-2 and dry protocol-3, respectively. The concluding pH value after employing dry protocol-3 was 6.8, indicating an acidic environment.

Figure 4.3 presents the pH variations observed in soil sample S3 following different treatment protocols. The plot clearly illustrates that the pH of soil S3 experienced an

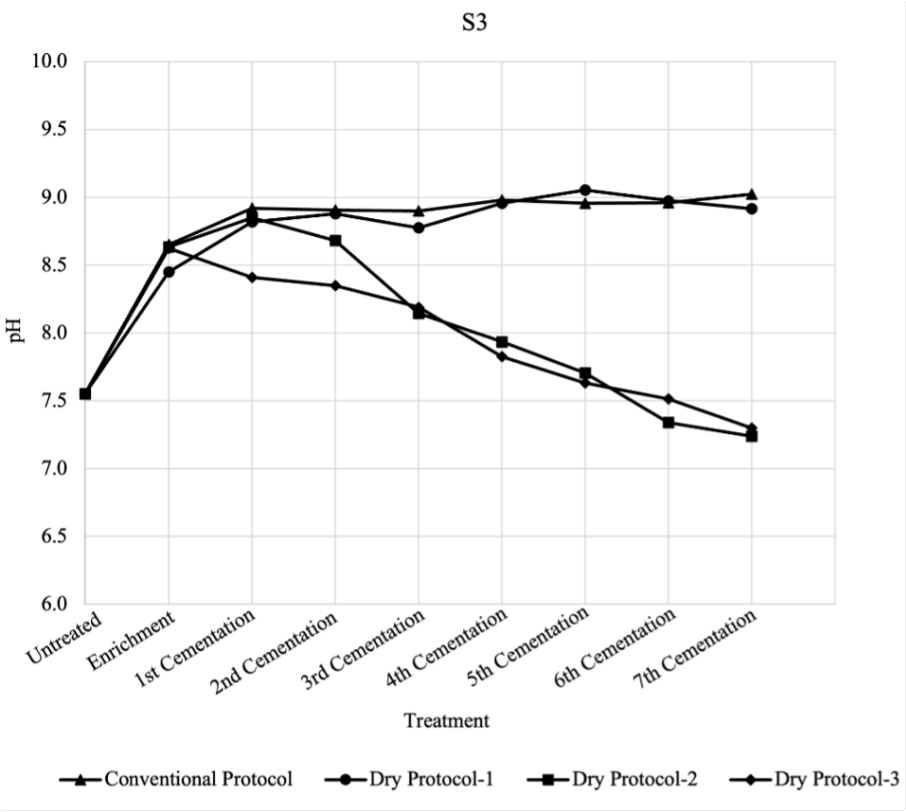

Figure 4.3: Change in pH of soil S3 with treatment rounds.

increase from its initial control value of 7.6 to approximately 8.6 after the stimulation process, indicating the soil's readiness for subsequent cementation treatments. In the case of the conventional protocol, the pH of the soil exhibited a consistent rise throughout the treatment rounds, eventually reaching a value of 9. Conversely, for dry protocol-1, the pH displayed minor fluctuations (both increases and decreases) after each treatment round, ultimately settling at a value of 8.9 upon completion of the treatments. Notably, for dry protocol-2 and dry protocol-3, a distinct and steep pH gradient emerged after the second round of cementation, which persisted until the conclusion of the experiment. Consequently, both protocols yielded a final pH value of approximately 7.3.

Figure 4.4 illustrates the pH dynamics observed in soil sample S4 under the influence of the four protocols during successive treatment rounds. Analyzing the plot, it is evident that the pH values surged to 8.3 and 8.6 following the stimulation treatment for the three dry protocols and the conventional protocol, respectively. For the conventional protocol, the soil's pH experienced an initial increase over the first two rounds of cementation, followed by a decline until the fourth round. Subsequently, there was another increase after the fifth round, and finally, a slight decrease led to a pH value of 8.1 by the end of the experiment. In contrast, dry protocol-1 exhibited the highest pH values among all four protocols. After the stimulation treatment, the pH rose from 8.3 to 9.3 after the first round, with a minor decrease in the subsequent round. It then maintained a relatively stable pH level, eventually settling at 8.9 at the conclusion of the experiment. Similarly, dry protocol-2 and dry protocol-3 demonstrated comparable pH patterns throughout the treatment of soil sample S4. For dry protocol-2, the pH reached 8.5 after the second round of treatment, followed by a gradual decrease to 7.8 by the end of the experiment. Dry protocol-3 increased the soil's pH to 8.9 after the second round, and subsequently followed a similar pattern to dry protocol-2 after the third round of cementation treatment.

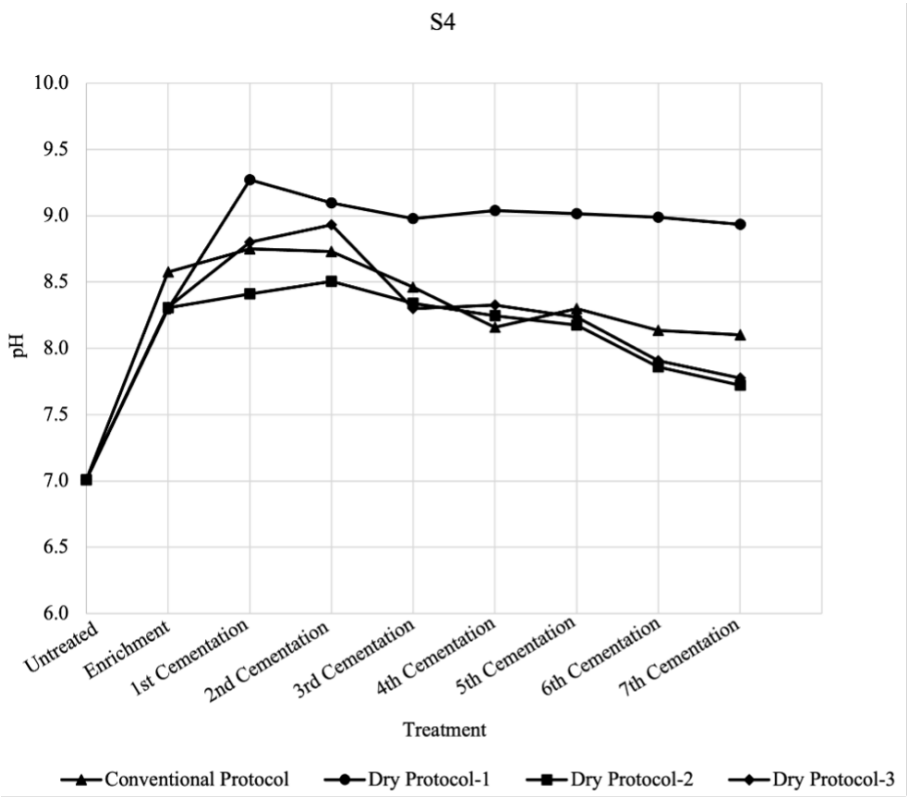

Figure 4.4: Change in pH of soil S4 with treatment rounds.

Figure 4.5 presents the pH fluctuations observed in soil sample S5 across various treatment rounds using different protocols. Prior to any treatment, the soil exhibited an acidic pH of 6.5. Following the stimulation treatment, the pH levels significantly rose to 8.3 for the conventional protocol and approximately 8 for the dry protocols. In the case of the conventional protocol, the soil's pH experienced a gradual increase until the fourth round of cementation, followed by a slight decrease, ultimately reaching a value of 9. Similarly, for dry protocol-1, the pH reached a comparable value to the conventional protocol at the end of the treatment. However, dry protocol-2 demonstrated an initial pH increase up to the second round of cementation, but experienced a sharp decline thereafter, resulting in a final pH value of 7.1. For dry

protocol-3, the pH initially increased to 8.7, but gradually decreased as the rounds of cementation progressed, leading to a final pH value of 8.4.

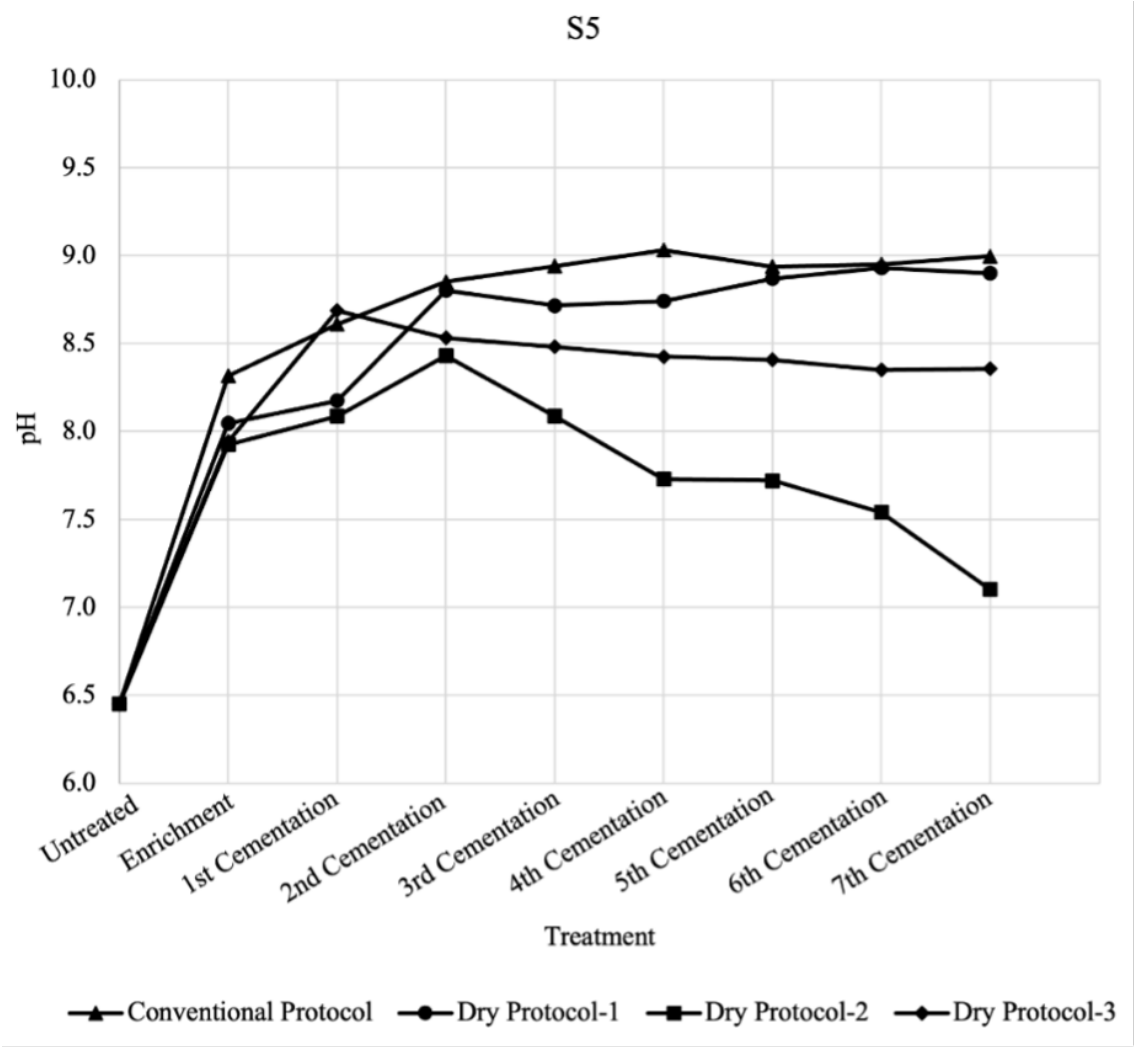

Figure 4.5: Change in pH of soil S5 with treatment rounds.

4.2 Calcium Carbonate ($CaCO_3$) Precipitation

4.2.1 Effect of Protocols on Soils

Figures 4.6 to 4.9 presented below depict a series of graphs illustrating the impact of different protocols, namely the Conventional Protocol, Dry Protocol-1, Dry Protocol-2, and Dry Protocol-3, on the five soil samples utilized in this study. These graphs effectively illustrate the variations in calcium carbonate precipitation resulting from each protocol for every soil type after the initial, third, fifth, and seventh iterations of the cementation treatment process.

Figure 4.6 illustrates the impact of the conventional protocol on the soils during four distinct stages of the cementation treatment process. Notably, the graph demonstrates a gradual increase in Calcite precipitation across all soil types as the cementation treatment advances. This observed increase can be attributed to the introduction and subsequent mixing of the cementation solution with the soil samples. The range of $CaCO_3$ precipitation resulting from the conventional protocol varied from 3.28% for soil S1, characterized by minimal fines, to 4.8% for soil S3.

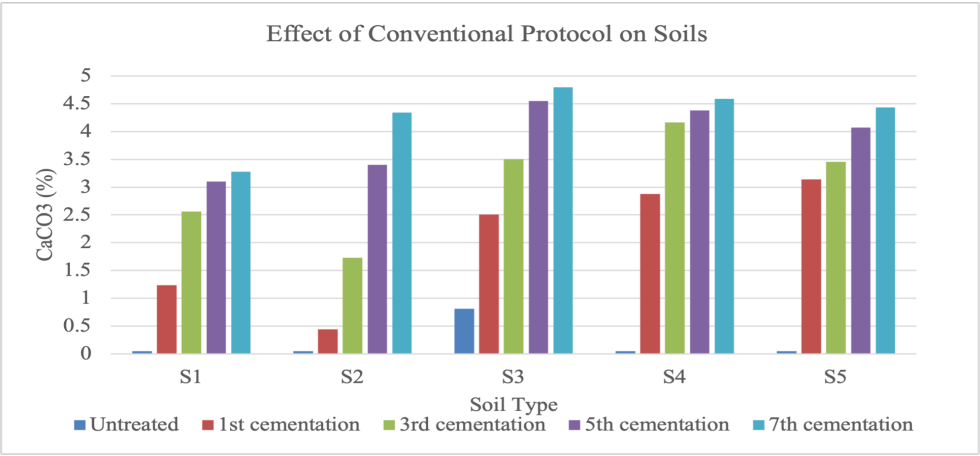

Figure 4.6: Effect of Conventional Protocol on Soils.

The second graph exhibits the efficacy of Dry Protocol-1 on all five soil samples. Figure 4.7 illustrates that the pattern of calcium carbonate increment is analogous to that observed in the conventional protocol. In both cases, an increase in calcium carbonate is observed after each successive round of treatment. However, it is noteworthy that the calcite precipitation resulting from Dry Protocol-1 consistently falls below that of the conventional protocol.

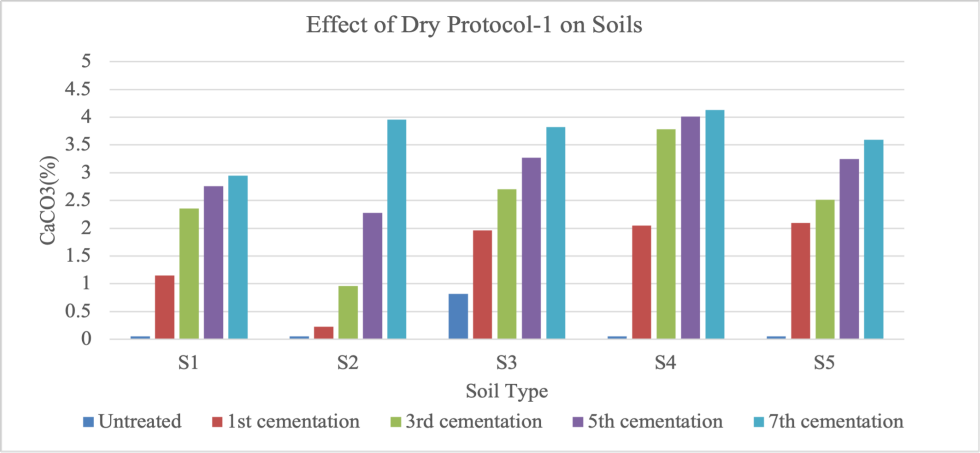

Figure 4.7: Effect of Dry Protocol-1 on Soils.

The third plot within Figure 4.8 illustrates the response of the soils to Dry Protocol-2 as the cycles of cementation progress. It is evident that following the initial round of cementation, there is a substantial increase in calcium carbonate or calcite precipitation across all five soil samples. This notable increase can be attributed to the stimulation of natural bacteria, which received ample nutrients at the commencement of the cementation cycles, thereby triggering significant calcium carbonate production.

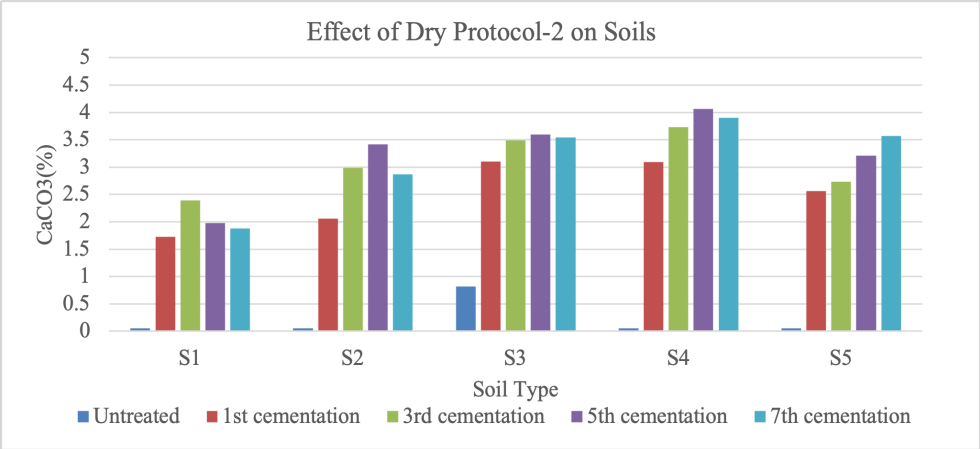

Figure 4.8: Effect of Dry Protocol-2 on Soils.

Furthermore, the plot reveals that, except for soil S5, the calcium carbonate precipitation decreases after increasing. This can be attributed to the specific characteristics of Dry Protocol-2, which involves the addition of deionized (DI) water without incorporating it into the soil sample during successive cementation cycles. Consequently, the calcium carbonate precipitates within the soil remain undisturbed, leading to a decrease in soil permeability. As a result, the DI water added in subsequent rounds is unable to penetrate the soil in all directions effectively.

This limitation may have caused further calcium carbonate precipitation to be restricted primarily to the upper part of the soil sample, as the DI water is unable to permeate throughout. Consequently, there is a loss of calcite when replacing the solution in the soil samples after each round, thereby explaining the observed decrease in calcite percentage during subsequent rounds.

Figure 4.9 depicts the calcium carbonate precipitation resulting from Dry Protocol-3 across all five soil types. The observed precipitation patterns in the soils following the first round of treatment closely resemble those observed with Dry Protocol-2. No-

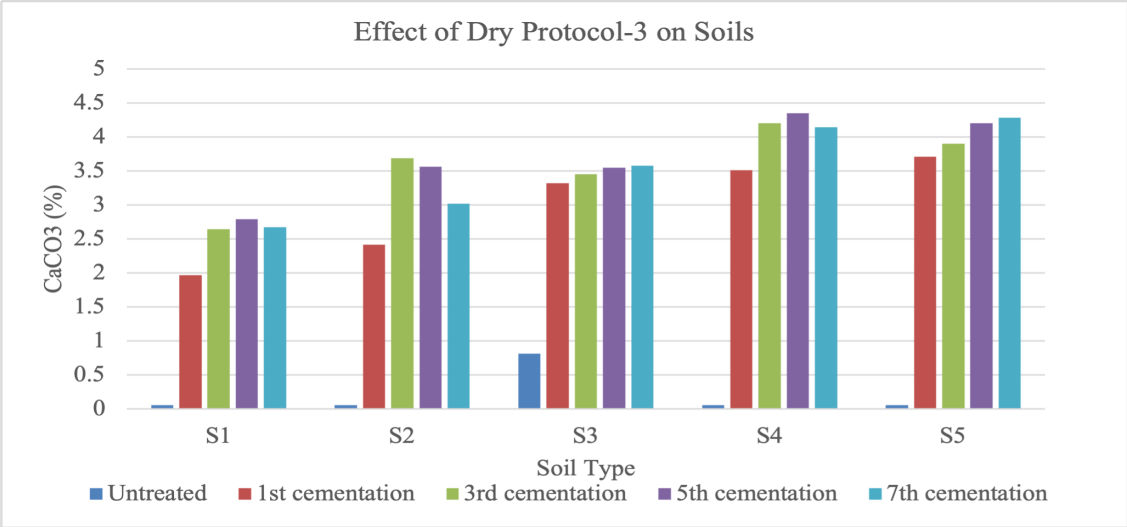

Figure 4.9: Effect of Dry Protocol-3 on Soils

tably, there is a decrease in calcite percentage in soils S1, S2, and S4 after increase.

This observed decrease can be attributed to the subsequent treatment rounds, where DI water is added and mixed with the soil samples. Since the calcite precipitation is initially high, the introduction of DI water and subsequent mixing may lead to the breakdown and removal of the formed calcite when replacing the solution with fresh DI water. In other words, the mixing action during successive treatment cycles may disrupt the existing calcite, causing its removal from the soil sample. This phenomenon provides a plausible explanation for the decrease in calcite percentage observed in soils after the increases.

4.2.2 Effect on Soils across Protocols

Figures 4.10 through 4.14 provide valuable insights into the calcium carbonate precipitation resulting from the four treatments applied to individual soils. These figures can serve as a useful resource to comprehend and determine the most effective protocol for each specific soil sample.

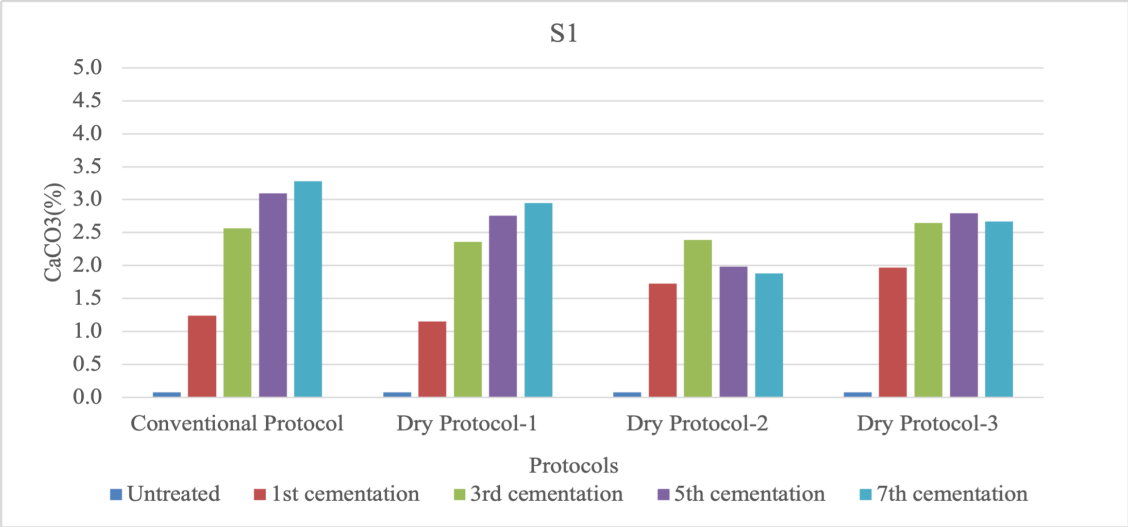

Figure 4.10: $CaCO_3$ Precipitation from different protocols on Soil S1

Figure 4.10 exhibits the calcium carbonate precipitation in soil sample S1 throughout various stages of cementation rounds, employing the four protocols. An examination of the results reveals that the conventional protocol yielded 3.28%, the highest accumulation of $CaCO_3$ at the conclusion of the seven treatment rounds. Dry protocol-1 ranked second with 2.95%, generating the second-highest amount of $CaCO_3$ in soil S1. Dry protocol-3 and dry protocol-2 secured the third and fourth positions with 2.67% and 1.88%, respectively, in terms of overall precipitation by the end of the treatment rounds, although the initial round of treatment under these protocols resulted in significantly higher precipitation. Therefore, if the objective is to achieve the maximum calcium carbonate precipitate, the conventional protocol emerges as the optimal choice. However, if the target is to produce approximately 3% of calcium carbonate, dry protocol-1 can be considered. On the other hand, if a precipitation level about 2.5% is desired after the third round of treatment, dry protocol-2 or dry protocol-3 could be suitable options.

Figure 4.11 presents the calcium carbonate precipitation in soil sample S2 under different protocols. Analysis of the results reveals that the conventional protocol yielded the highest calcium carbonate precipitation, reaching 4.3% at the conclusion of the treatment. Similarly, dry protocol-1 generated a precipitation of 3.9% by the end of the treatment. Dry protocol-2 exhibited a precipitation of 3.42% after the fifth round of treatment, which decreased to 2.87% at the conclusion of the treatment. Likewise, although dry protocol-3 initially produced approximately 3.69% of calcium carbonate after the third round, the final precipitate at the end of the treatment reduced to 3.02%. This reduction in precipitate can be attributed to reduced bacterial activity and the steps involved in the protocol. Hence, if the goal is to obtain a calcium carbonate precipitate exceeding 4%, and there is sufficient time to complete all the treatment rounds, the conventional protocol should be selected. However, if a precipitation level of around 3.9% is sufficient, dry protocol-1 can be employed. Dry protocol-2 and dry protocol-3 demonstrated precipitations of 2.06% and 2.42%, respectively, which were the highest among all protocols after the first round of treatment. Additionally, dry protocol-2 produced the same percentage as the conventional protocol after the fifth round. Similarly, dry protocol-3 exhibited a precipitation of 3.69% after the third round of treatment. Therefore, depending on the desired percentage of calcium carbonate precipitation and time constraints, one of the four protocols can be chosen accordingly.

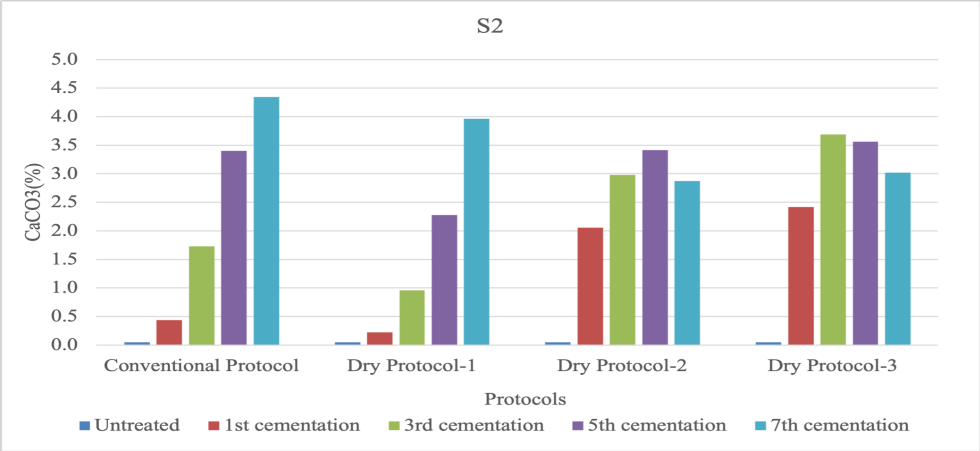

Figure 4.11: $CaCO_3$ Precipitation from different protocols on Soil S2

Figure 4.12 illustrates the calcium carbonate precipitation in soil sample S3 resulting from different protocols. It is evident that precipitation increases with each treatment round for all four protocols, with only a slight decrease observed at the end of treatment for dry protocol-2. It is worth noting that the untreated soil already contained 0.81% of calcium carbonate. Among the protocols, the conventional protocol yielded the highest calcium carbonate precipitate, reaching 4.8% at the conclusion of the treatment. Dry protocol-1, dry protocol-2, and dry protocol-3 produced precipitates of 3.82%, 3.54%, and 3.58%, respectively, at the end of the treatment. Significant increases in precipitation were observed for both the conventional protocol and dry protocol-1 as the treatment progressed. However, dry protocol-2 and dry protocol-3 demonstrated a substantial initial jump in precipitation from 0.81% to slightly above 3% after the first round of treatment. As the treatment rounds continued, the precipitation exhibited only marginal increments.

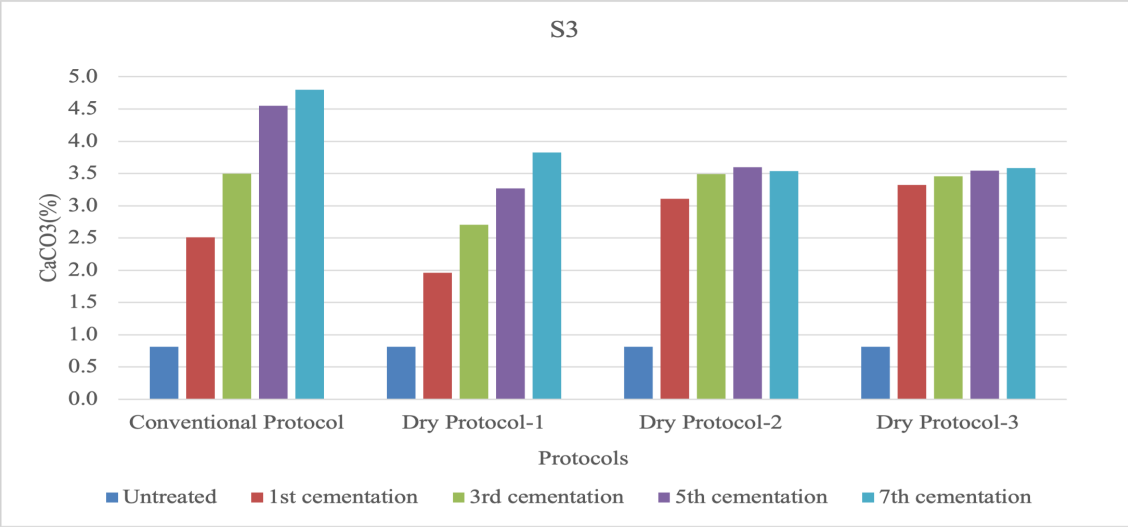

Figure 4.12: $CaCO_3$ Precipitation from different protocols on Soil S3

Figure 4.13 provides insights into the calcium carbonate precipitation in soil sample S4 resulting from the four protocols. The highest percentage of precipitation, 4.59%, was observed with the conventional protocol at the conclusion of the seven treatment rounds. Notably, the calcium carbonate precipitation exhibited a continuous increase with each round of treatment for the conventional protocol. Similarly, dry protocol-1 demonstrated increasing precipitation after each treatment round, ultimately reaching a value of 4.13%. Dry protocol-2 and dry protocol-3 displayed a similar pattern, with approximately 3.09% and 3.59% of calcium carbonate precipitating after the first cementation round, respectively. The precipitation steadily increased for both protocols, reaching values of 4.06% and 4.35% after the fifth round, before experiencing a slight reduction. Ultimately, at the end of the seven rounds of cementation, the precipitate levels reached 3.9% and 4.15% for dry protocol-2 and dry protocol-3, respectively.

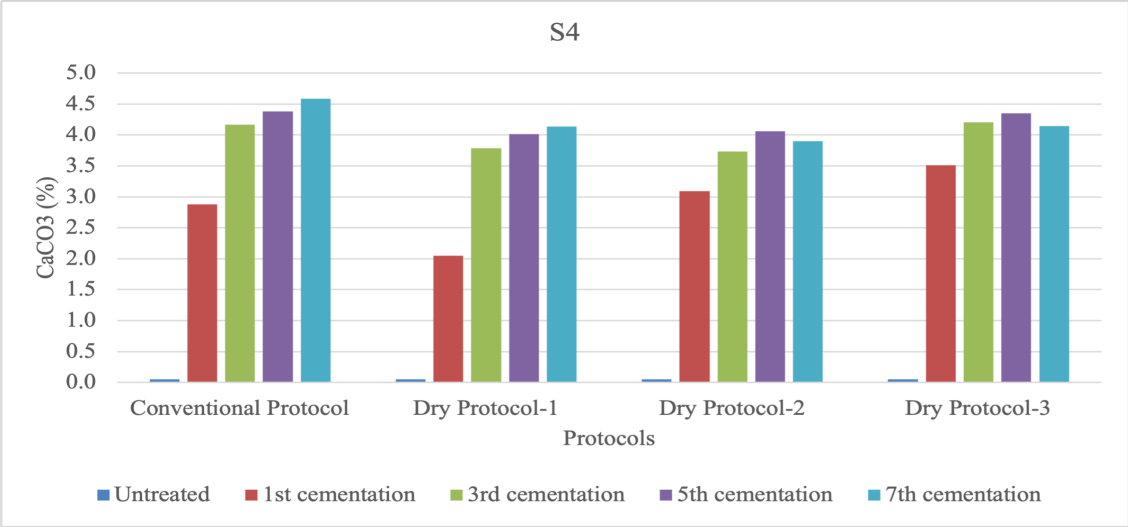

Figure 4.13: $CaCO_3$ Precipitation from different protocols on Soil S4

Figure 4.14 illustrates the response of soil sample S5 to different protocols across various treatment rounds. Unlike the other soils examined in this study, the graph demonstrates a consistent increase in calcium carbonate precipitation for all four protocols from the first round of treatment to the final round. Among the applied protocols, the conventional protocol yielded the highest precipitate, measuring 4.43% at the conclusion of the treatment rounds. Compared to all protocols, dry protocol-1 initially displayed relatively lower effectiveness in terms of precipitation for soil S5 during the early treatment rounds. However, it caught up and achieved a precipitate of 3.59% by the end of the treatment process, which was comparable to the value obtained from dry protocol-2. Dry protocol-3 exhibited the highest calcite precipitation after the first round of treatment, amounting to 3.71%. However, there was only a marginal increase in calcite precipitation afterward, resulting in a final value of 4.29% after the last round of treatment.

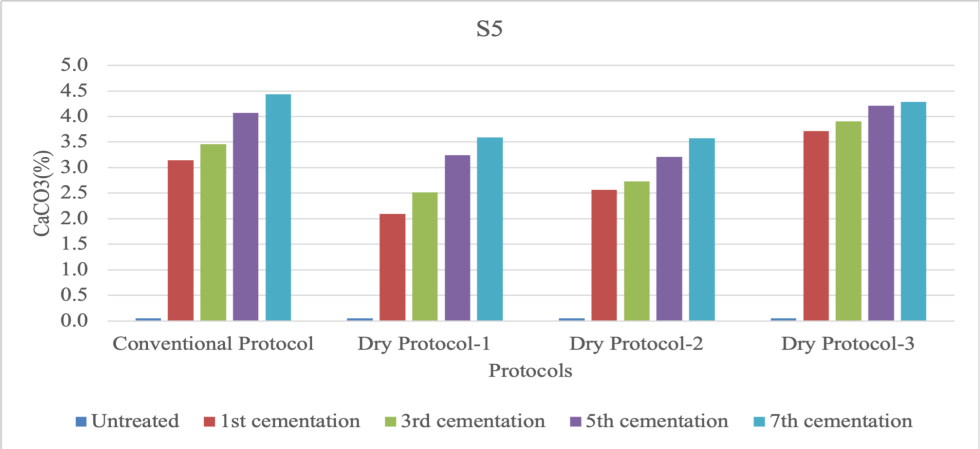

Figure 4.14: $CaCO_3$ Precipitation from different protocols on Soil S5

4.2.3 Analysis for Each Round of Cementation

Based on the preceding analysis, it is evident that the wet protocol demonstrated excellent performance for all the soils investigated in this study after seven rounds of cementation. However, the performance of each protocol varied considerably after each treatment round. Figure 4.15 provides valuable insights, indicating that dry protocol-3 exhibited the most promising results across all soils after the first round of treatment, making it a suitable choice when aiming for approximately 2% calcium carbonate precipitation.

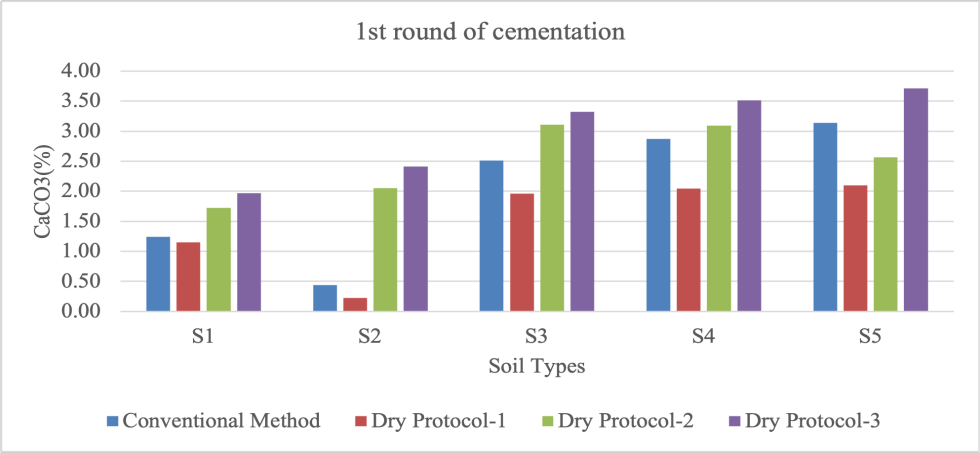

Figure 4.15: $CaCO_3$ Precipitation after 1st Round of Cementation

After three treatment rounds, as we can see in Figure 4.16 dry protocol-3 continued to lead, although the other protocols were catching up. Notably, in soil S3, both the conventional protocol and dry protocol-2 surpassed the calcium carbonate production achieved by dry protocol-3 after the third round. Subsequently, after five rounds, as we can see in Figure 4.17 the precipitation attributed to dry protocol-3 remained relatively stable, while the other protocols witnessed significant increases, particularly the conventional protocol, which exhibited a substantial boost in calcium carbonate precipitation. Finally, in Figure 4.18, after completing all seven treatment rounds, the wet protocol emerged as the most effective across all soil types. Dry protocol-1 followed as the second most successful protocol for all soils except soil S5. Dry protocol-3 ranked third in terms of precipitation for all soils, but it secured the second highest precipitation in soil S5. Conversely, dry protocol-2 resulted in the lowest precipitation across all soil types.

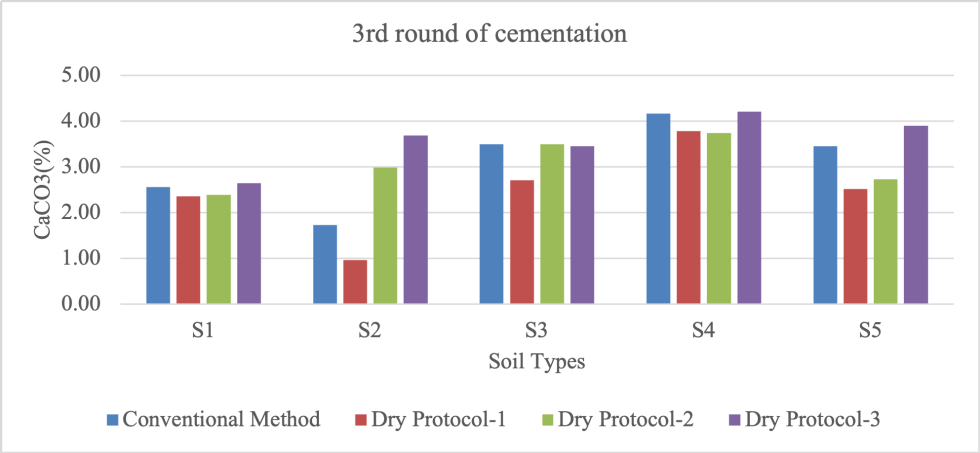

Figure 4.16: $CaCO_3$ Precipitation after 3rd Round of Cementation

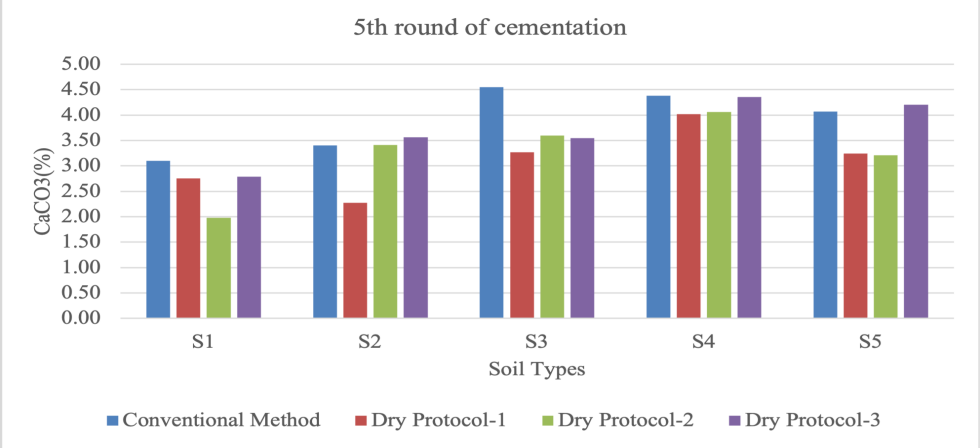

Figure 4.17: $CaCO_3$ Precipitation after 5th Round of Cementation

Therefore, the selection of a specific protocol should consider the desired amount of calcium carbonate precipitation and the available time frame for conducting the treatment process.

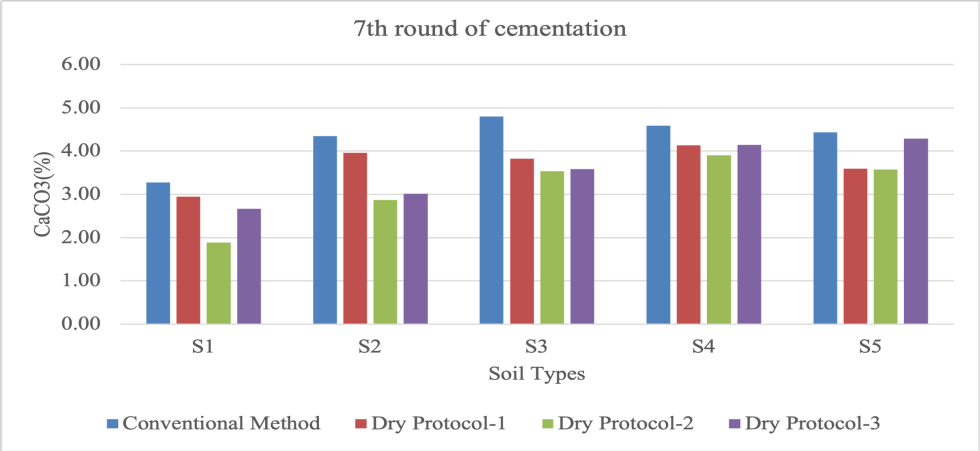

Figure 4.18: $CaCO_3$ Precipitation after 7th Round of Cementation

4.2.4 Free Swell Index

The test results revealed a direct correlation between calcium carbonate precipitation and the reduction in soil swelling potential. Two plastic soils, namely S4 and S5, underwent a free swell test upon completion of the treatment process. It was observed from Figure 4.19 that the conventional protocol yielded the highest calcite precipitation in both S4 and S5 after seven rounds of treatment. Additionally, the treated soil samples exhibited the lowest free swell index when treated with the conventional protocol.

Specifically, for soil S4, the free swell index decreased from the control value of 50% to 18.18% with the conventional protocol, indicating a significant reduction in swelling. Similarly, for soil S5, the free swell index decreased from the control value of 150% to 79.67% when treated with the conventional protocol.

Dry protocol-3, dry protocol-1, and dry protocol-2 ranked as the second, third, and fourth protocols, respectively, in terms of calcium carbonate precipitation in both S4 and S5. The corresponding free swell indexes for S4 and S5 after treatment

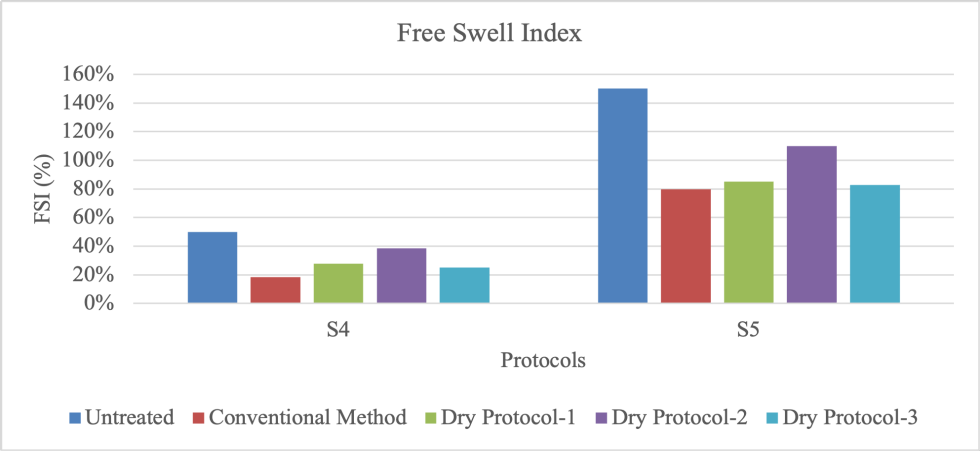

Figure 4.19: Comparison of Free Soil Index before Treatment and After seven Rounds of Treatment

were 25% and 82.69% for dry protocol-3, 27.73% and 85% for dry protocol-1, and 38.32% and 110% for dry protocol-2. These results indicate that all four protocols effectively reduced soil swelling, with the conventional protocol demonstrating the most favorable outcomes.

CHAPTER 5:

STATISTICAL ANALYSIS

Statistical analysis plays a crucial role in research, offering valuable insights from collected data. It provides an unbiased framework for decision-making, enabling us to move beyond the subjective interpretations and instead rely on statistical evidence to substantiate our conclusions. In our work, statistical analysis is employed to assess the impact of protocol, round of cementation and soil type in the experiment. It involves the application of suitable statistical test, which in our case, is Analysis of Variance (ANOVA), to identify any statistically significant differences or relationships among the treatment groups. By quantifying and evaluating the observed effects, statistical analysis helps us understand the implications of the treatments and draw valid conclusions about their effectiveness.

5.1 Experimental Design and Statistical Model

Experimental design pertains to the statistical discipline concerned with formulating and assessing experiments. It encompasses the methodologies utilized in agriculture, medicine, biology, engineering, and industrial production (Palmer nd). The goal of experimental design is to maximize the quality and efficiency of the experiment, ensuring that the collected data is reliable and informative. It is essential because it allows researchers to control variables, establish cause-and-effect relationships, and

minimize the influence of irrelevant factors. By employing randomization and replication techniques, experimental design ensures that the collected data is statistically valid, precise, and accurate.

In our experiment, we utilize a repeated measures design, which involves measuring the same subject at multiple time points. We chose this design to effectively capture temporal information. By measuring subjects at different time points, the repeated measures design enables us to observe and analyze temporal dynamics, such as changes in $CaCO_3$ precipitation over time. In our statistical analysis, we decide to choose multiple two-way ANOVA instead of a higher order ANOVA. This choice is motivated by our preference for a simpler and more easily interpretable model. Analyzing each factor independently will yield results that are straightforward and easier to communicate. Also, it is our interest to understand Protocols (1st factor) and Rounds of Treatment (2nd factor) pairwise and not just as a whole. This requires us to do post-hoc ANOVA analysis as well. Since Post-ANOVA analysis is generally considered simpler in lower-order ANOVA compared to more complex design such as higher-order ANOVA, we decide to choose multiple two-way ANOVA as our model. However, it is important to acknowledge that there may be some information loss concerning the interaction effect when employing multiple two-way ANOVA instead of higher-order ANOVA.

5.2 Analysis of Variance (ANOVA)

Analysis of Variance (ANOVA) is a statistical technique used to check if the means of two or more groups are significantly different from each other. It examines the influence of one or more factors by comparing the means of distinct samples (Singh nd).

ANOVA helps to assess whether the observed differences in sample means are larger than what would be expected due to random chance alone. The main objective of ANOVA is to determine if there is evidence to support the presence of a significant effect of one or more factors on a particular outcome variable. ANOVA analyzes the variation in the data and decomposes it into different sources, such as the variability within each group and the variability between groups. It quantifies the amount of variation explained by the factors of interest relative to the total variation observed in the data.

In our study, we examine the impact of three factors, namely Soil Type, Protocol, and Round of Treatments, on our response variable, $CaCO_3$ precipitation. To analyze the data, we employ ANOVA in a repeated design experimental setup, treating the "Round of Treatments" as a repeated measure. We then conduct two-way ANOVA twice, first with protocol fixed and second with soil type fixed.

ANOVA uses F-tests to statistically assess the equality of means when you have three or more groups (Frost nd). The F statistics used in F-test is defined as the ration of variance due to the difference in group to the variance due to a random chance.

$$F = \frac{Variance\ due\ to\ group\ differences}{Variance\ due\ to\ random\ chance} \tag{5.1}$$

This can also be expressed as

$$F = \frac{Variance\ between\ groups}{Variance\ within\ groups} \tag{5.2}$$

For a one-way ANOVA, we can further narrow it down to

$$F = \frac{MSB}{MSW} \tag{5.3}$$

where MSB represents Mean Sum of Squares between groups and MSW denotes Mean Sum of Squares within groups. MSB is defined as the ration of Sum of Squares Between the groups and Degrees of freedom between the group and is represented as

$$MSB = \frac{SSB}{DFB} \tag{5.4}$$

MSW, on the other hand, is defined as the ratio of Sum of Squares between the groups and Degrees of Freedom between the group and is represented as

$$MSW = \frac{SSW}{DFW} \tag{5.5}$$

DFB and DFW are Degrees of Freedom Between and Degree of Freedom Within respectively and are represented as

$$DFB = k - 1 \tag{5.6}$$

$$DFW = N - k \tag{5.7}$$

where k is the number of groups (or levels) and N is the total number of participants

across all groups (or levels). SSB and SSW can be calculated as

$$SSB = \Sigma\frac{(\Sigma x)^2}{n} - \frac{(\Sigma\Sigma x)^2}{N} \tag{5.8}$$

$$SSW = (\Sigma\Sigma x)^2 - \Sigma\frac{(\Sigma x)^2}{n} \tag{5.9}$$

where x is a measurement on individual of a group, n is a sample size within a group. Once the F-statistic is calculated based on the above calculations, we find the p-value from an F distribution using F-statistics, DFB and DFW. We then decide to reject or not reject our null hypothesis based on our p-value and pre-determined significance level, α. The Source table for ANOVA is shown in Table 5.1.

Table 5.1: ANOVA Source Table

Source of Variation	**Sum of Squares**	**Degrees of Freedom**	**Mean Squares**	**F Values**	**P-value**
Between Groups	SSB	DFB=k-1	MSB	MSB/MSW	From Upper tail $F_{k-1,N-k}$
Error	SSW	DFW=N-k	MSW		
Total	SST	N-1			

In our study, we use the statistical software SAS to generate the source table directly. Following the ANOVA analysis, if our hypothesis is rejected, we conduct a

post-ANOVA analysis to examine differences among all possible group combinations. For this purpose, we employ Tukey's HSD (Honest Significant Difference) test as our chosen post hoc test method. This test is favored due to its simplicity, ability to facilitate comprehensive pairwise comparisons, and effective control of the Type I error rate.

5.3 Post ANOVA Analysis using Tukey's HSD

Tukey's HSD (Honest Significant Difference) test is a commonly employed post hoc test for evaluating the significance of mean differences between pairs of groups. Typically used as a subsequent analysis to one-way ANOVA, it serves as a follow-up when the F-test indicates a significant difference among at least some of the examined groups (User nd). The primary objective of Tukey's HSD test is to identify which specific pairs of group means significantly differ from each other, while controlling for the overall experiment-wise error rate. By accounting for multiple comparisons, Tukey's HSD test helps mitigate the issue of inflated Type I error (false positive) that can occur when conducting numerous pairwise comparisons without appropriate adjustments. The test procedure involves calculating a critical value, referred to as the HSD value, which is based on the residual mean square error obtained from the ANOVA. The HSD value represents the minimum significant difference required between two group means for them to be considered statistically different. To perform the test, pairwise comparisons are made between all possible combinations of group means. The absolute difference between each pair of means is compared to the HSD value. If the absolute difference exceeds the HSD value, the pair is deemed to have a significant difference at a predetermined level of significance (usually $\alpha = 0.05$).

In our study, Tukey's HSD test was specifically employed to examine the impact of

various rounds, treatments (protocols), and soil types on $CaCO_3$ precipitation. Unlike ANOVA, this test allows us to identify which specific groups of protocols, rounds, and soils exhibit statistically significant differences compared to other groups within their respective categories.

5.4 Analysis using Two-Way ANOVA

We conduct two-way ANOVA tests twice. The first test involves Protocol and Round of Treatments as factors, with Soil fixed. The second test involves Soil and Round of Treatments as factors, with Protocol fixed.

5.4.1 Two-Way ANOVA with Soils as Constant

We perform a two-way ANOVA analysis with the factors Protocol and Round of Treatments, while keeping the Soil fixed. Since we have five different types of soil, we will conduct five separate sets of tests within this two-way ANOVA framework, each test focusing on a specific soil type. This approach allows us to examine the combined effects of Protocol and Round of Treatments on each soil type individually. Under this scheme, we will have three different sets of hypotheses:

1. Hypothesis I

   Null Hypothesis (H_o): Protocol does not have a significant effect on the $CaCO_3$ precipitation, regardless of the Round of Treatments and with Soil fixed, indicating that different protocols do not lead to different outcomes.

   Alternative Hypothesis (H_A): Protocol has a significant effect on the $CaCO_3$ precipitation, independent of the Round of Treatments and with Soil fixed, indicating that different protocols lead to different outcomes.

2. Hypothesis II

Null Hypothesis (H_o): Round of Treatments does not have a significant effect on the $CaCO_3$ precipitation, independent of Protocol and with Soil fixed, indicating that varying the treatment rounds do not lead to different outcomes.

Alternative Hypothesis (H_A): Round of Treatments has a significant effect on the $CaCO_3$ precipitation, independent of Protocol and with Soil fixed, indicating that varying the treatment rounds lead to different outcomes.

3. Hypothesis III

   Null Hypothesis (H_o): There is no interaction effect between the Protocol and Round of Treatments on the $CaCO_3$ precipitation when the Soil is fixed, indicating that the combined effect of the factors is simply the sum of their individual effects.

   Alternative Hypothesis (H_A): There is an interaction effect between the Protocol and Round of Treatments on the $CaCO_3$ precipitation, with soil fixed, implying that the combined effect of the factors differs from what would be expected based on their individual effects alone. This suggests that the relationship between the factors is not simply additive.

5.4.1.1 Two-Way ANOVA with soil S1 as constant

We perform two-way ANOVA keeping soil S1 as constant. We set the significance level to 0.05. We get the source table as shown in Table 5.2.

Conclusion: The p-value for the Protocol (treatment) is 0.0494 which is less than our significance level of $\alpha(0.05)$. Therefore, we reject our 1st null hypothesis and conclude that the effect of at least one of the protocols is different from rest of the others for soil S1.

Table 5.2: ANOVA Source Table for soil S1

Effect	**Numerator Degree of Freedom**	**Denominator Degree of Freedom**	**F Values**	**P-value**
Protocol	3	4	6.64	0.0494
Round	3	12	33.67	$<$0.0001
Protocol $\times$*Round*	9	12	4.54	0.0087

The p-value for the Round is less than 0.0001 which is also less than our significance level of α (0.05). Therefore, we reject our 2nd null hypothesis and conclude that the effect of at least one of the rounds is different from rest of the others for soil S1. The p-value for the interaction between Protocol and Round is 0.0087, which is below our significance level ($\alpha = 0.05$). Thus, we reject the null hypothesis and conclude that there is a significant interaction effect between Protocol and Round of Treatments on $CaCO_3$ precipitation for soil S1. This indicates that the combined effect of these factors differs from what would be anticipated based on their individual effects alone.

Since the null hypotheses are rejected, we perform post-ANOVA analysis using Tukey's HSD to see which all protocols, rounds and protocol-round interactions differ from each other. The results of Tukey's HSD test are shown in Figure 5.1 and Figure 5.2.

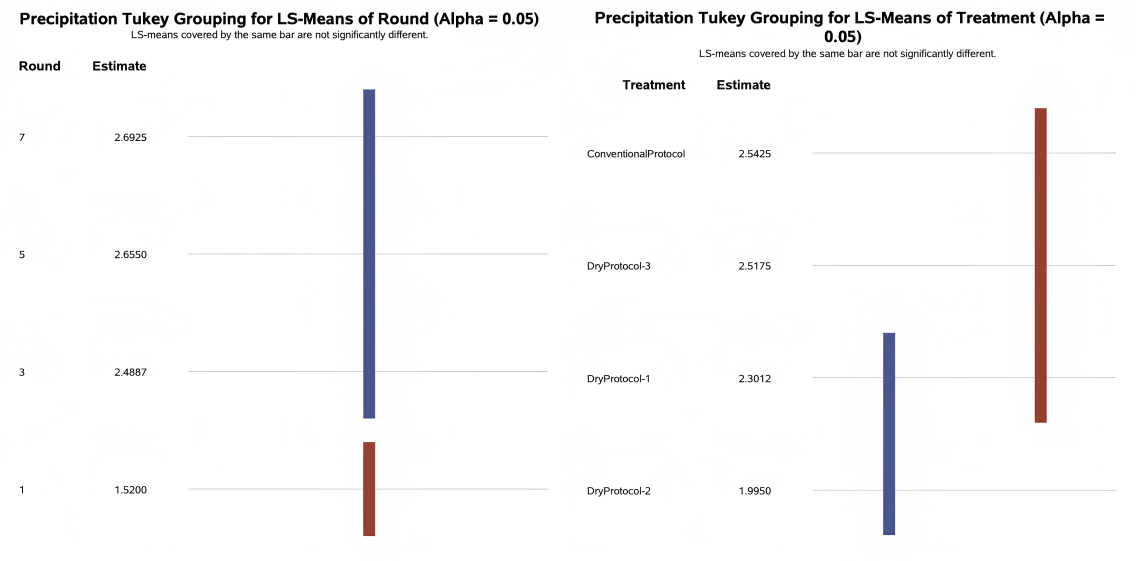

Figure 5.1: Tukey's HSD for Soil S1
Round (Left) and Treatment/Protocol (Right)

The results presented in the left chart of Figure 5.1 indicate that, in the case of soil S1, the levels of $CaCO_3$ precipitation in rounds 3, 5, and 7 are not statistically different regardless of the protocol used. However, the precipitation in round 1 differs significantly from rounds 3, 5, and 7.

On the other hand, the right chart in Figure 5.1 demonstrates that, irrespective of the number of rounds, Conventional Protocol, Dry Protocol-3, and Dry Protocol-1 exhibit no significant differences for soil S1. Additionally, Dry Protocol-1 and Dry Protocol-2 are not significantly different from each other, but Dry Protocol-2 significantly differs from the remaining protocols.

The results in Figure 5.2 presents the interaction between rounds and protocols for soil S1.

The top-left chart in Figure 5.2 shows that for round 1, Conventional Protocol is not significantly different from Dry Protocol-2; Dry Protocol-2 and Dry Protocol-

3 are not significantly different; Conventional Protocol and Dry Protocol-1are not significantly different. Except for these three combinations, all other combinations of round and protocol are significantly different.

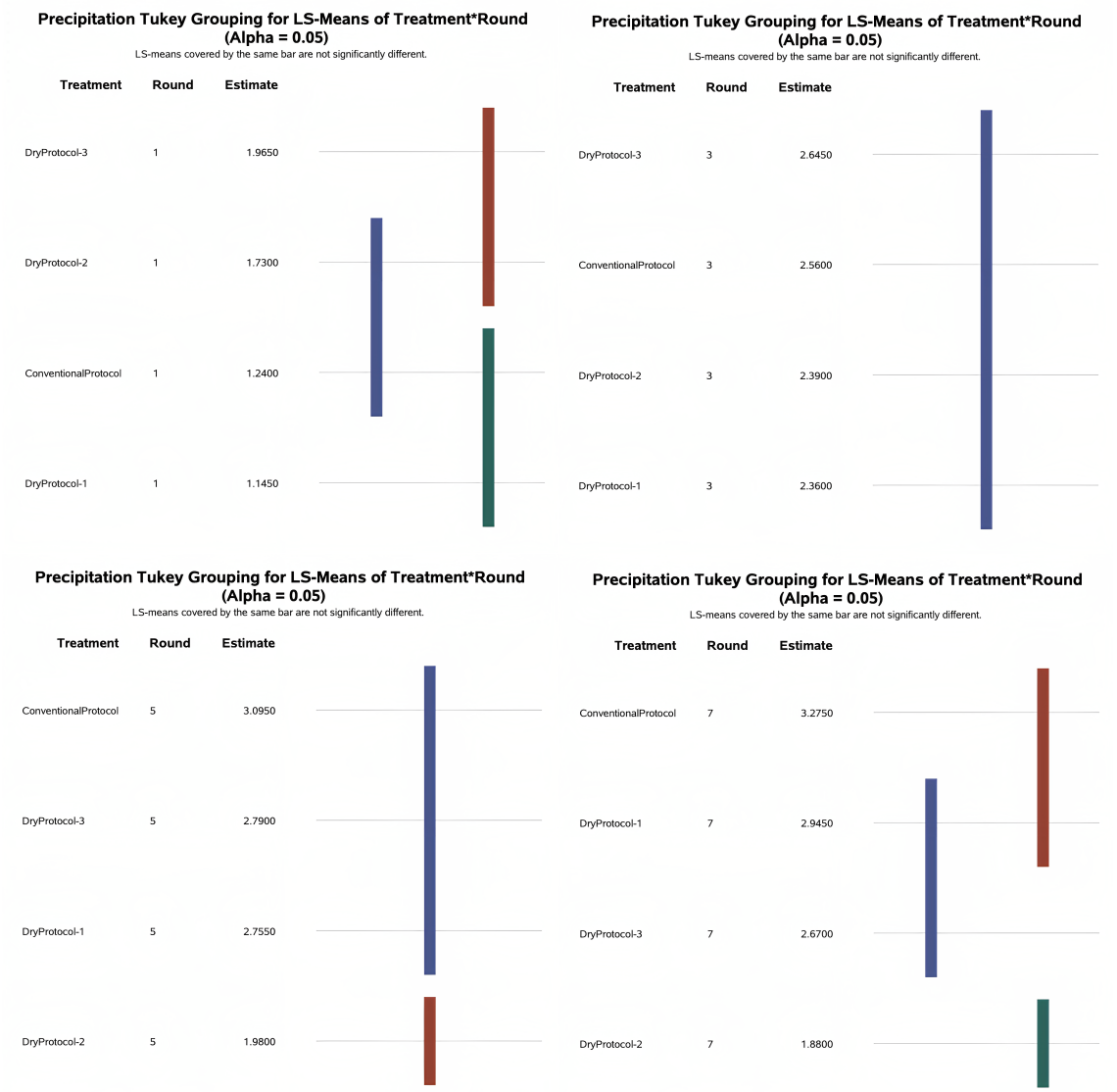

Figure 5.2: Tukey's HSD for Soil S1 Round and Protocol Interaction

The top-right chart in Figure 5.2 shows that for round 3, none of the protocols

are statistically different from each other. The bottom-left chart in Figure 5.2 shows that for round 5, Conventional Protocol, Dry Protocol-1 and Dry-Protocol-3 are not statistically different from each other but Dry Protocol-2 is statistically different from rest of the protocols.

The bottom-right chart in Figure 5.2 shows that for round 7, Dry Protocol-1 and Dry Protocol-3 are not statistically different from each other; Conventional Protocol and Dry Protocol-1 are not statistically different from each other. Except for these combinations, all other two combinations of rounds and protocols are statistically different.

5.4.1.2 Two-way ANOVA with soil S2 as Constant

We perform two-way ANOVA keeping soil S2 as constant. We set the significance level (α) to 0.05. We get the source table as shown in Table 5.3.

Table 5.3: ANOVA Source Table for soil S2

Effect	**Numerator Degree of Freedom**	**Denominator Degree of Freedom**	**F Values**	**P-value**
Protocol	3	4	15.28	0.0117
Round	3	12	111.35	$<$0.0001
Protocol $\times$*Round*	9	12	21.62	$<$0.0001

Conclusion: The p-value for the Protocol (treatment) is 0.0117 which is less than our significance level of α (0.05). Therefore, we reject our 1st null hypothesis and conclude that the effect of at least one of the protocols is different from rest of the others for soil S2. The p-value for the Round is less than 0.0001 which is also less than our significance level of α (0.05). Therefore, we reject our 2nd null hypothesis and conclude that the effect of at least one of the rounds is different from rest of the others for soil S2. The p-value for the interaction between Protocol and Round is 0.0087, which is below our significance level ($\alpha = 0.05$). Thus, we reject the null hypothesis and conclude that there is a significant interaction effect between Protocol and Round of Treatments on $CaCO_3$ precipitation for soil S2. This indicates that the combined effect of these factors differs from what would be anticipated based on their individual effects alone.

Since the null hypotheses are rejected, we perform post-ANOVA analysis using Tukey's HSD to see which all protocols, rounds and protocol-round interactions differ from each other. The results of Tukey's HSD test are shown in Figure 5.3 and Figure 5.4.

The results presented in the left chart of Figure 5.3 indicate that, in the case of soil S2, the levels of $CaCO_3$ precipitation in each round is statistically different regardless of the protocol used.

On the other hand, the right chart in Figure 5.3 demonstrates that, irrespective of the number of rounds, for S2, effect of Conventional Protocol and Dry Protocol-2 are not significantly different; effect of Dry Protocol-2 and Dry Protocol-3 are not significantly different. Except for these combination, all other two combinations of protocols are significantly different from each other.

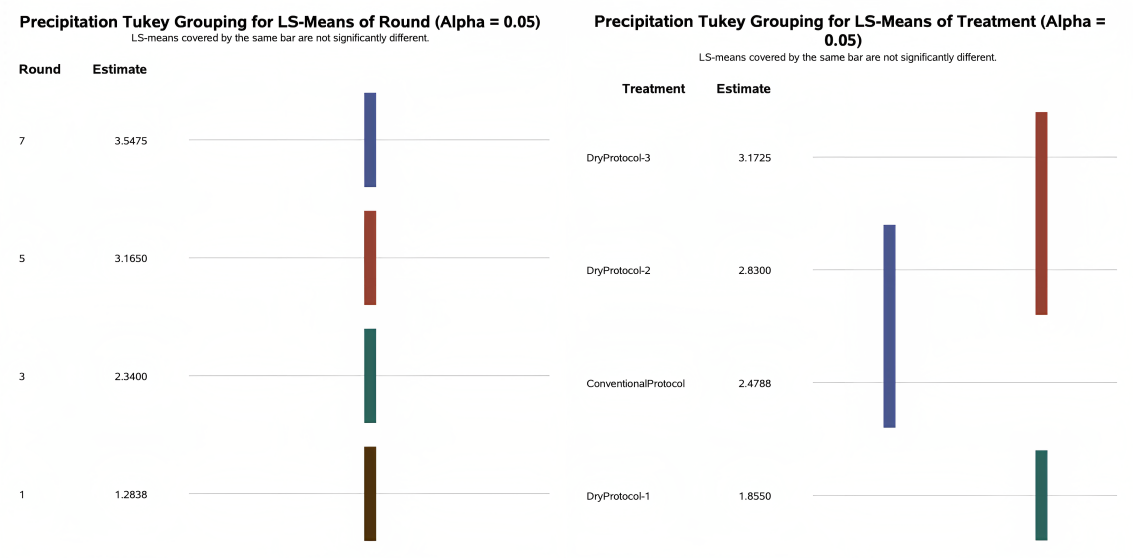

Figure 5.3: Tukey's HSD for Soil S2
Round (Left) and Treatment/Protocol (Right)

The results in Figure 5.4 presents the interaction between rounds and protocols for soil S2.

The top-left chart in Figure 5.4 shows that for soil S2, for round 1, Conventional Protocol is not significantly different from Dry Protocol-1; Dry Protocol-2 and Dry Protocol-3 are not significantly different; but both Conventional Protocol and Dry Protocol-1 are significantly different from each of Dry Protocol-2 and Dry Protocol-3.

The top-right chart in Figure 5.4 shows that for soil S2, for round 3, each of the protocols is statistically different from other protocols. The bottom-left chart in Figure 5.4 shows that for round 5, Conventional Protocol, Dry Protocol-2 and Dry-Protocol-3 are not statistically different from each other, but Dry Protocol-1 is statistically different from rest of the protocols.

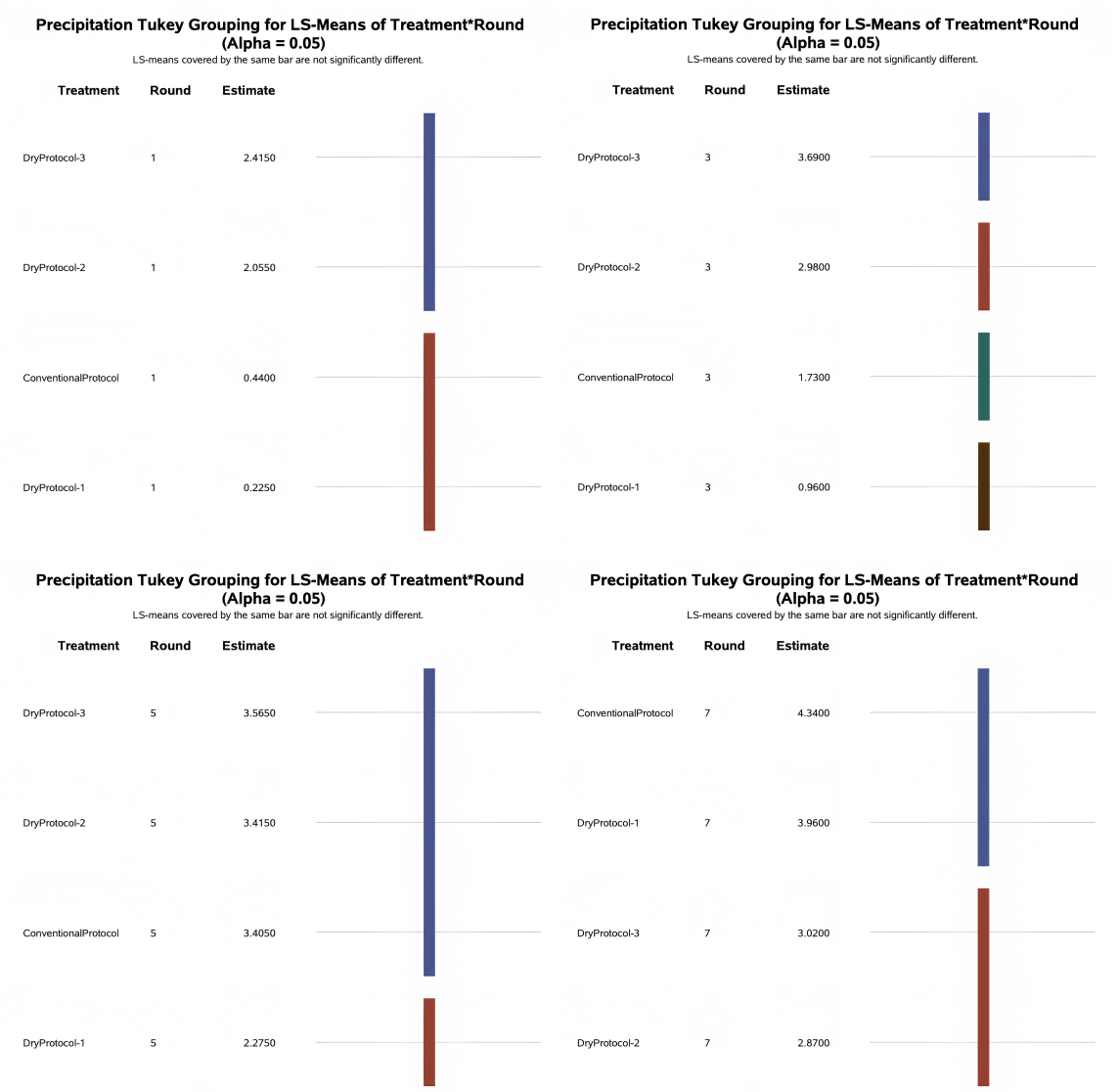

Figure 5.4: Tukey's HSD for Soil S2 Round and Protocol Interaction

The bottom-right chart in Figure 5.4 shows that, for soil S2, for round 7, Conventional Protocol and Dry Protocol-1 are not statistically different from each other; Dry Protocol-2 and Dry Protocol-3 are not statistically different from each other, but Conventional Protocol and Dry Protocol-1 are both statistically different from each

of Dry Protocol-2 and Dry Protocol-3.

5.4.1.3 Two-way ANOVA with soil S3 as constant

We perform two-way ANOVA keeping soil S3 as constant. We set the significance level (α) to 0.05. We get the source table as shown in Table 5.4.

Table 5.4: ANOVA Source Table for soil S3

Effect	**Numerator Degree of Freedom**	**Denominator Degree of Freedom**	**F Values**	**P-value**
Protocol	3	4	74.42	0.0006
Round	3	12	123.86	<0.0001
Protocol ×*Round*	9	12	22.69	<0.0001

Conclusion: The p-value for the Protocol (treatment) is 0.0006 which is less than our significance level of α (0.05). Therefore, we reject our 1st null hypothesis and conclude that the effect of at least one of the protocols is different from rest of the others for soil S3. The p-value for the Round is less than 0.0001 which is also less than our significance level of α (0.05). Therefore, we reject our 2nd null hypothesis and conclude that the effect of at least one of the rounds is different from rest of the others for soil S3. The p-value for the interaction between Protocol and Round is less than 0.0001, which is also below our significance level ($\alpha = 0.05$). Thus, we reject

the null hypothesis and conclude that there is a significant interaction effect between Protocol and Round of Treatments on $CaCO_3$ precipitation for soil S3. This indicates that the combined effect of these factors differs from what would be anticipated based on their individual effects alone.

Since the null hypotheses are rejected, we perform post-ANOVA analysis using Tukey's HSD to see which all protocols, rounds and protocol-round interactions differ from each other. The results of Tukey's HSD test are shown in Figure 5.5 and Figure 5.6.

The results presented in the left chart of Figure 5.5 indicate that, in the case of soil S3, the levels of $CaCO_3$ precipitation in each round is statistically different regardless of the protocol used.

On the other hand, the right chart in Figure 5.5 demonstrates that, irrespective of the number of rounds, for soil S3, effect of Dry Protocol-2 and Dry Protocol-3 are not significantly different but both Dry Protocol-2 and Dry Protocol-3 are statistically different from each of Conventional Protocol and Dry Protocol-1; also, the effect of Conventional Protocol significantly differs from Dry Protocol-1.

The results in Figure 5.6 presents the interaction between rounds and protocols for soil S3.

The top-left chart in Figure 5.6 shows that for soil S3, for round 1, effect of Dry Protocol-3 and Dry Protocol-2 on $CaCO_3$ precipitation are not statistically different but effect of all other two combination of protocol and round are statistically different.

The top-right chart in Figure 5.6 shows that for soil S3, for round 3, effect of Conventional Protocol, Dry Protocol-2, and Dry Protocol-3 are not significantly different but Dry Protocol-1's effect is significantly different from effect of other protocols.

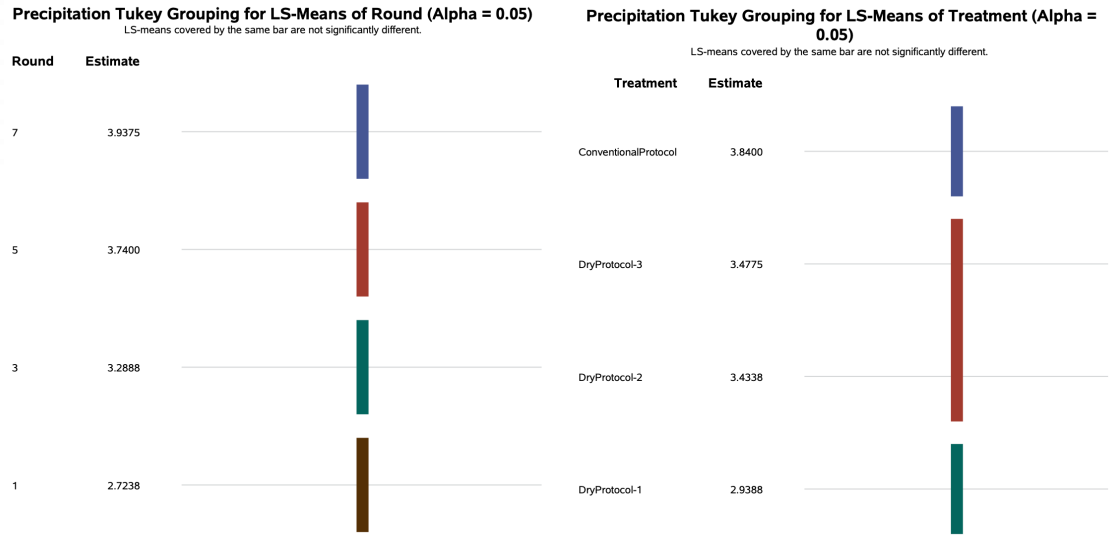

Figure 5.5: Tukey's HSD for Soil S3
Round (Left) and Treatment/Protocol (Right)

The bottom-left chart in Figure 5.6 shows that for round 5, effect of Dry Protocol-2 and Dry Protocol-3 are not statistically different from each other; effect of Dry Protocol-1 and Dry Protocol-3 are not statistically different from each other but effect of all other two combination of protocol and round are statistically different.

The bottom-right chart in Figure 5.6 shows that for round 7, effect of Dry Protocol-1 and Dry Protocol-3 are not statistically different from each other; effect of Dry Protocol-2 and Dry Protocol-3 are not statistically different from each other but effect of all other two combination of protocol and round are statistically.

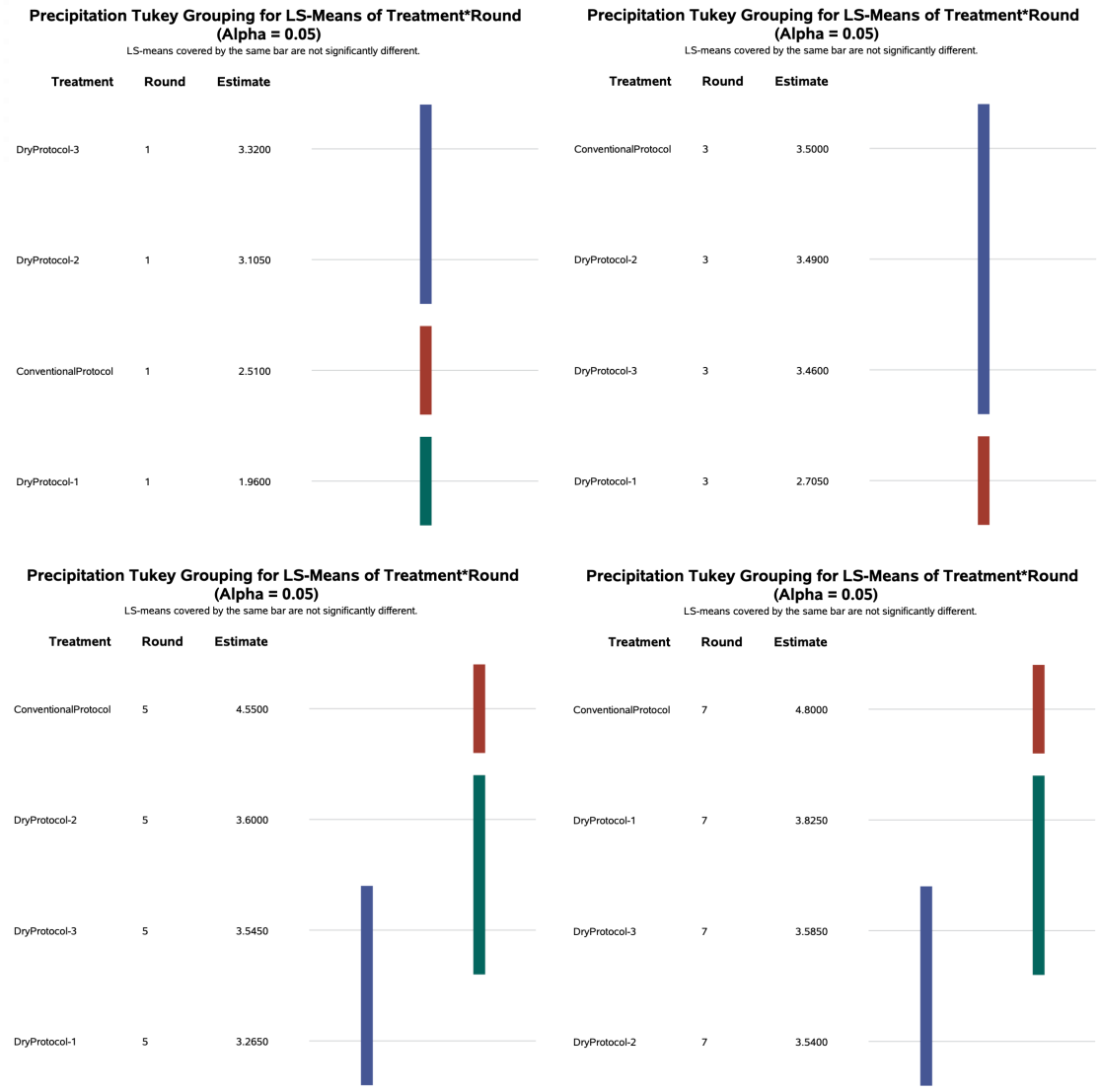

Figure 5.6: Tukey's HSD for Soil S3 Round and Protocol Interaction

5.4.1.4 Two-way ANOVA with soil S4 as constant

We perform two-way ANOVA keeping soil S4 as constant. We set the significance level (α) to 0.05. We get the source table as shown in Table 5.5.

Table 5.5: ANOVA Source Table for soil S4

Effect	**Numerator Degree of Freedom**	**Denominator Degree of Freedom**	**F Values**	**P-value**
Protocol	3	4	1.58	0.3269
Round	3	12	26.16	$<$0.0001
Protocol $\times$*Round*	9	12	1.99	0.1331

Conclusion: The p-value for the Protocol (treatment) is 0.3269 which is greater than our significance level of α (0.05). Therefore, we do not reject our 1st null hypothesis and conclude that the effect of at least one of the protocols is not different from rest of the others for soil S4. The p-value for the Round is less than 0.0001 which is also less than our significance level of α (0.05). Therefore, we reject our 2nd null hypothesis and conclude that the effect of at least one of the rounds is different from rest of the others for soil S4. The p-value for the interaction between Protocol and Round is 0.1331, which is above our significance level ($\alpha = 0.05$). Thus, we do not reject the null hypothesis and conclude that there is no significant interaction effect between Protocol and Round of Treatments on $CaCO_3$ precipitation for soil S4. This indicates that the combined effect of these factors does not differs from what would be anticipated based on their individual effects alone.

Since the null hypothesis for round effect is rejected, we perform post-ANOVA

analysis using Tukey's HSD to see which rounds differ from each other. Unlike rest of the results for soil S1, S2 and S3, we do not perform post-ANOVA analysis for protocol effect and round-protocol interaction effect because we fail to reject our null hypothesis I and III. The result of Tukey's HSD test is shown in Figure 5.7.

The result presented in the Figure 5.7 indicate that, in the case of soil S4, regardless of the protocol, the effect of round 3, round 5 and round 7 are not statistically different but round 1 is statistically different from rest of the rounds.

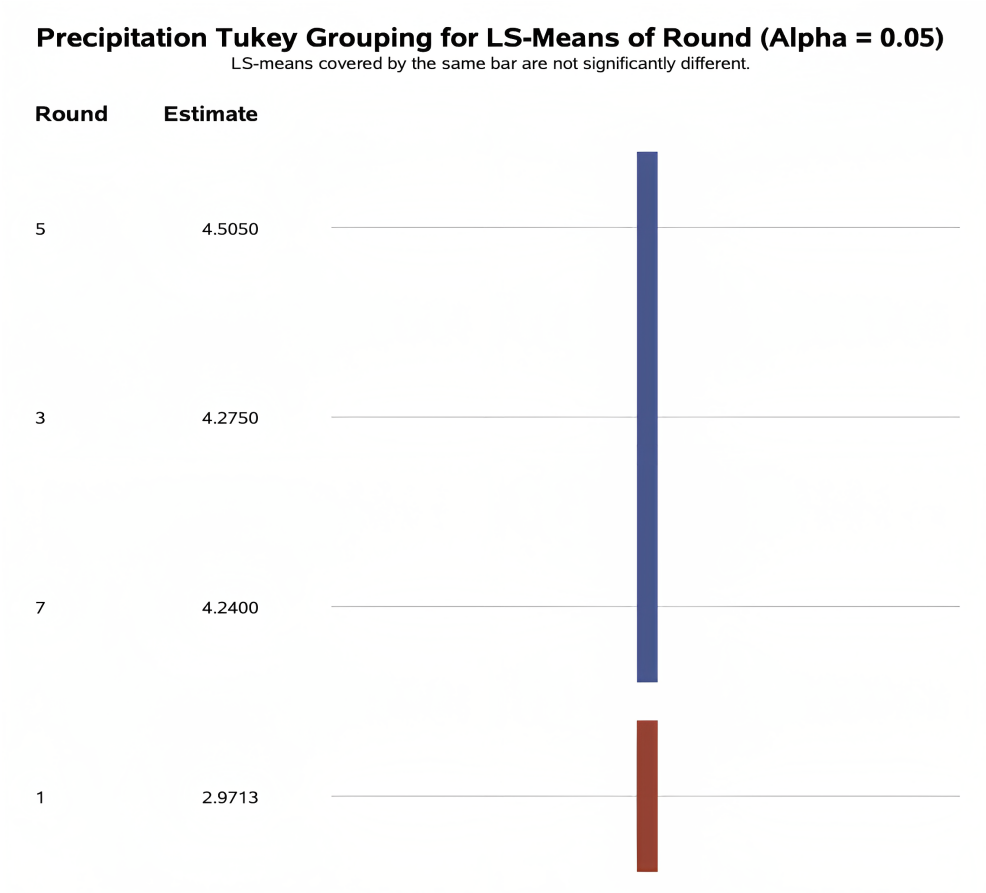

Figure 5.7: Tukey's HSD for Soil S4 Round Effect

5.4.1.5 Two-way ANOVA with soil S5 as constant

We perform two-way ANOVA keeping soil S5 as constant. We set the significance level (α) to 0.05. We get the source table as shown in Table 5.6.

Table 5.6: ANOVA Source Table for soil S5

Effect	**Numerator Degree of Freedom**	**Denominator Degree of Freedom**	**F Values**	**P-value**
Protocol	3	4	38.58	0.0021
Round	3	12	42.76	$<$0.0001
Protocol $\times$*Round*	9	12	1.46	0.2644

Conclusion: The p-value for the Protocol (treatment) is 0.0021 which is less than our significance level of α (0.05). Therefore, we reject our 1st null hypothesis and conclude that the effect of at least one of the protocols is different from rest of the others for soil S5. The p-value for the Round is less than 0.0001 which is also less than our significance level of α (0.05). Therefore, we reject our 2nd null hypothesis and conclude that the effect of at least one of the rounds is different from rest of the others for soil S5. The p-value for the interaction between Protocol and Round is 0.2644, which is above our significance level ($\alpha = 0.05$). Thus, we do not reject the null hypothesis and conclude that there is no significant interaction effect between Protocol

and Round of Treatments on $CaCO_3$ precipitation for soil S5. This indicates that the combined effect of these factors does not differs from what would be anticipated based on their individual effects alone.

Since the null hypothesis for round effect and protocol effect is rejected, we perform post-ANOVA analysis using Tukey's HSD to see which rounds and protocols differ from each other. The result of Tukey's HSD test is shown in Figure 5.8.

The results presented in the left chart of Figure 5.8 indicate that, in the case of soil S5, the levels of $CaCO_3$ precipitation in each round is statistically different regardless of the protocol used.

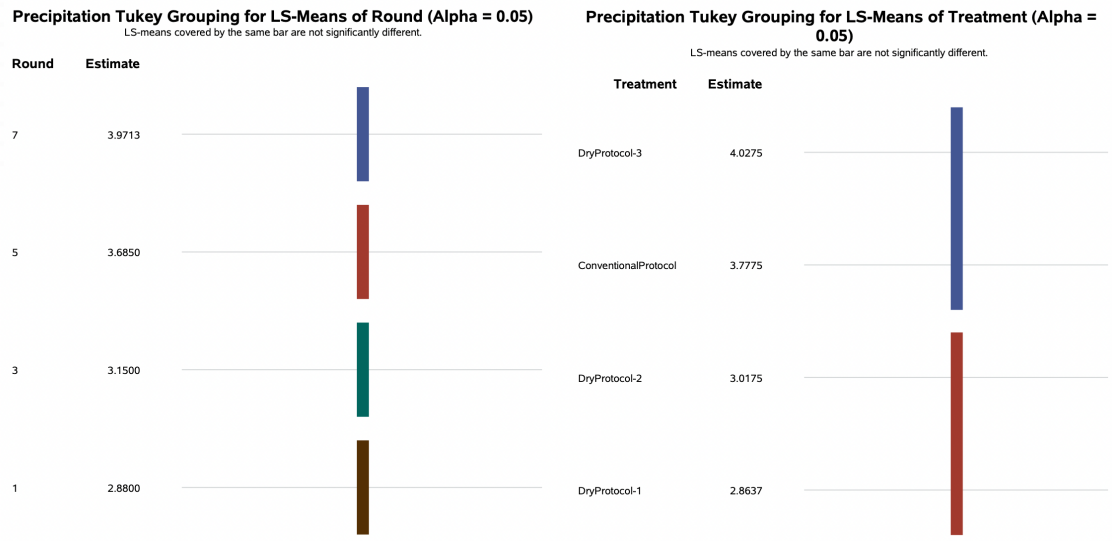

Figure 5.8: Tukey's HSD for Soil S5
Round (Left) and Treatment/Protocol (Right)

On the other hand, the right chart in Figure 5.8 demonstrates that, irrespective of the number of rounds, the effect of Dry Protocol-3 and Conventional Protocol on $CaCO_3$ precipitation is not statistically different; the effect of Dry Protocol-1 and Dry Protocol-2 on $CaCO_3$ precipitation is not significantly different; but each of Dry

Protocol-3 and Conventional Protocol is significantly different from Dry Protocol-1 and Dry Protocol-2.

5.4.2 Two-Way ANOVA with Protocol as Constant

We perform a two-way ANOVA analysis with the factors Soil Type and Round of Treatments, while keeping the Protocol fixed. Since we have four different types of protocols, we will conduct four separate sets of tests within this two-way ANOVA framework, each test focusing on a specific protocol. This approach allows us to examine the combined effects of Soil Type and Round of Treatments on each protocol individually. Under this scheme, we will have three different sets of hypotheses.

1. Hypothesis I

   Null Hypothesis (H_o): Soil Type does not have a significant effect on the $CaCO_3$ precipitation, independent of the Round of Treatments and with Protocol fixed, indicating that different soil type does not lead to different outcomes.

   Alternative Hypothesis (H_A): Soil Type has a significant effect on the $CaCO_3$ precipitation, independent of the Round of Treatments and with Protocol fixed, indicating that different protocols lead to different outcomes.

2. Hypothesis II

   Null Hypothesis (H_o): Round of Treatments does not have a significant effect on the $CaCO_3$ precipitation, independent of the Round of Treatments and with Protocol fixed, indicating that varying the treatment rounds do not lead to different outcomes.

   Alternative Hypothesis (H_A): Round of Treatments has a significant effect on the $CaCO_3$ precipitation, independent of the Round of Treatments and with

Protocol fixed, indicating that varying the treatment rounds lead to different outcomes.

3. Hypothesis III

   Null Hypothesis (H_o): There is no interaction effect between Soil Type and Round of Treatments on the $CaCO_3$ precipitation when the Protocol is fixed, indicating that the combined effect of the factors is simply the sum of their individual effects.

   Alternative Hypothesis (H_A): There is an interaction effect between the Soil Type and Round of Treatments on the $CaCO_3$ precipitation, with protocol fixed, implying that the combined effect of the factors differs from what would be expected based on their individual effects alone. This suggests that the relationship between the factors is not simply additive.

5.4.2.1 Two-Way ANOVA with Conventional Protocol as Constant

We perform two-way ANOVA keeping Conventional Protocol as constant. We set the significance level (α) to 0.05. We get the source table as shown in Table 5.7.

Conclusion: The p-value for the Soil Type is less than 0.0001 which is also less than our significance level of α (0.05). Therefore, we reject our 1st null hypothesis and conclude that the effect of at least one of the soil types is different from rest of the others for conventional protocol. The p-value for the Round is less than 0.0001 which is also less than our significance level of α (0.05).

Table 5.7: ANOVA Source Table for Conventional Protocol as constant

Effect	**Numerator Degree of Freedom**	**Denominator Degree of Freedom**	**F Values**	**P-value**
Soil Type	4	5	80.90	$<$0.0001
Round	3	15	26.16	$<$0.0001
Soil Type $\times$*Round*	12	15	16.66	$<$0.0001

Therefore, we reject our 2nd null hypothesis and conclude that the effect of at least one of the rounds is different from rest of the others for conventional protocol. The p-value for the interaction between Soil Type and Round is less than 0.0001, which is also below our significance level ($\alpha = 0.05$). Thus, we reject the null hypothesis and conclude that there is a significant interaction effect between Soil Type and Round of Treatments on $CaCO_3$ precipitation for conventional protocol. This indicates that the combined effect of these factors differs from what would be anticipated based on their individual effects alone.

Since the null hypotheses are rejected, we perform post-ANOVA analysis using Tukey's HSD to see which all soil type, rounds and soil type-round interactions differ from each other. The results of Tukey's HSD test are shown in Figure 5.9 and Figure 5.10.

The results presented in the left chart of Figure 5.9 indicate that, in the case of

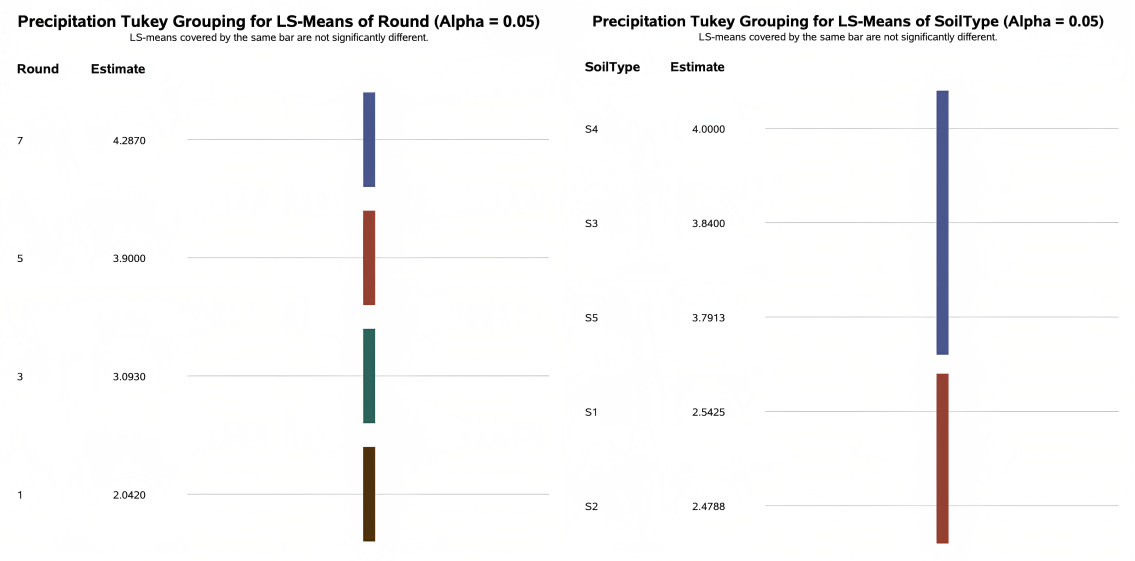

Figure 5.9: Tukey's HSD for Conventional Protocol Round (Left) and Soil Type (Right)

conventional protocol, the levels of $CaCO_3$ precipitation in each round is statistically different regardless of the type of soil used.

On the other hand, the right chart in Figure 5.9 demonstrates that, irrespective of the number of rounds, the effect of soil type on $CaCO_3$ precipitation is not statistically different for soil type S4, S3 and S5; the effect of soil type is not statistically different for soil type S1 and S2; but the effect of other combinations of soil types are statistically different.

The results in Figure 5.10 presents the interaction between soil type and rounds for conventional protocol.

The top-left chart in Figure 5.10 shows that for conventional protocol, for round 1, the effect of soil type S4 and soil type S5 are not statistically different; the effect of soil type S3 and S4 are not statistically different; but all other two combinations of soil type and round are significantly different from each other.

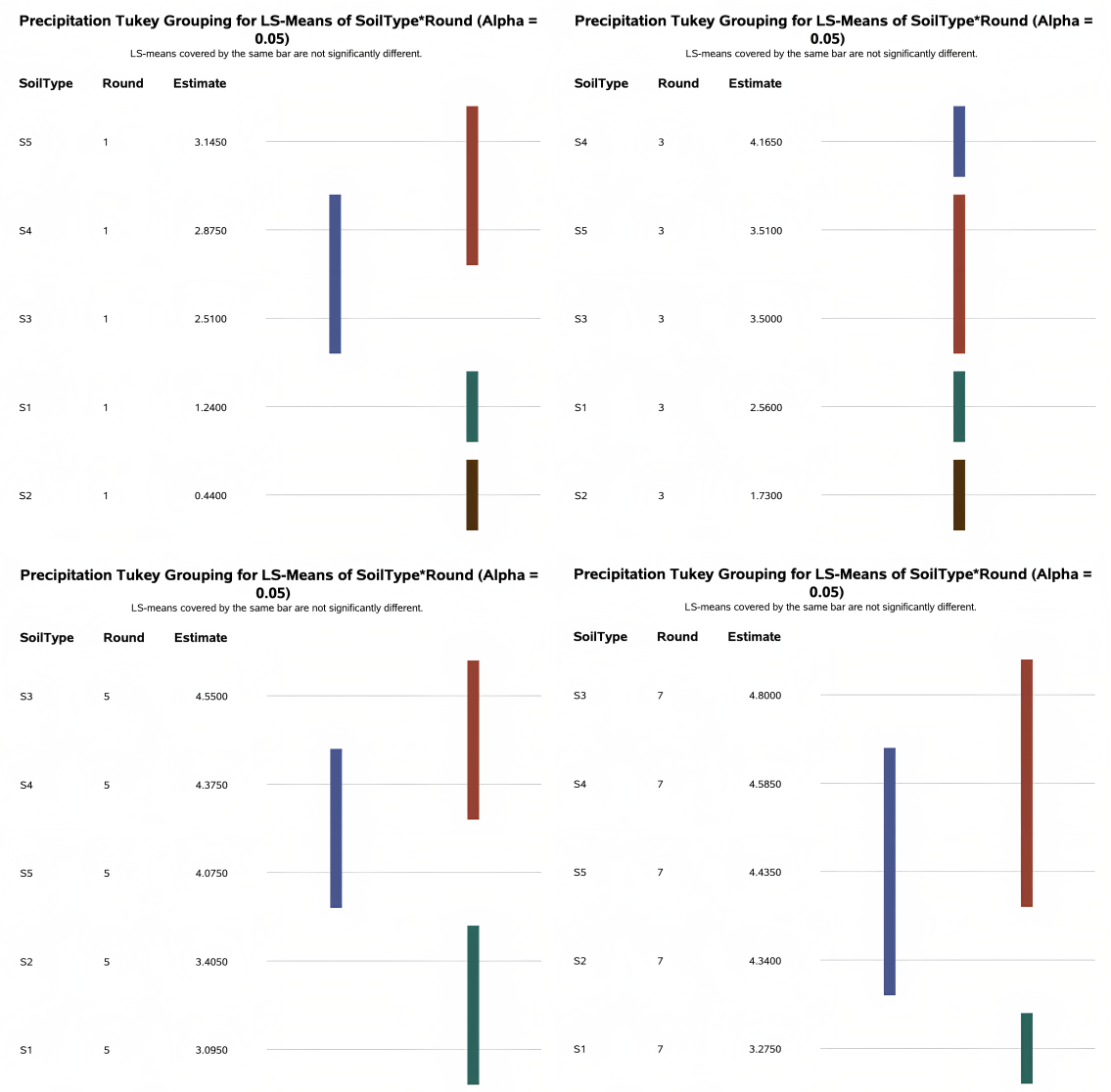

Figure 5.10: Tukey's HSD for Conventional Protocol Round and Soil Type Interaction

The top-right chart in Figure 5.10 shows that for conventional protocol, for round 3, the effect of soil type S3 and S5 are not statistically different; but all other two combinations of soil type and round are significantly different from each other.

The bottom-left chart in Figure 5.10 shows that for conventional protocol, for

round 5, the effect of soil type S3 and S4 are not statistically different; the effect of soil type S4 and S5 are not statistically different; the effect of soil type S1 and S2 are not statistically different; but all other two combinations of soil type and round are significantly different from each other.

The bottom-right chart in Figure 5.10 shows that for conventional protocol, round 7, the effect of soil type S3, S4 and S5 are not significantly different from each other; the effect of soil type S2, S4 and S5 are also not statistically different from each other; but the effect of soil type S1 is statistically different from rest of soils.

5.4.2.2 Two-Way ANOVA with Dry Protocol-1 as constant

We perform two-way ANOVA keeping Conventional Protocol as constant. We set the significance level (α) to 0.05. We get the source table as shown in Table 5.8.

Table 5.8: ANOVA Source Table for Dry Protocol-1 as constant

Effect	**Numerator Degree of Freedom**	**Denominator Degree of Freedom**	**F Values**	**P-value**
Soil Type	4	5	84.49	$<$0.0001
Round	3	15	109.57	$<$0.0001
Soil Type $\times$*Round*	12	15	5.98	$<$0.0009

Conclusion: The p-value for the Soil Type is less than 0.0001 which is also less

than our significance level of α (0.05). Therefore, we reject our 1st null hypothesis and conclude that the effect of at least one of the soil types is different from rest of the others for Dry Protocol-1. The p-value for the Round is less than 0.0001 which is also less than our significance level of α (0.05). Therefore, we reject our 2nd null hypothesis and conclude that the effect of at least one of the rounds is different from rest of the others for Dry Protocol-1. The p-value for the interaction between Soil Type and Round is less than 0.0001, which is also below our significance level (α = 0.05). Thus, we reject the null hypothesis and conclude that there is a significant interaction effect between Soil Type and Round of Treatments on $CaCO_3$ precipitation for Dry Protocol-1. This indicates that the combined effect of these factors differs from what would be anticipated based on their individual effects alone.

Since the null hypotheses are rejected, we perform post-ANOVA analysis using Tukey's HSD to see which all soil type, rounds and soil type-round interactions differ from each other. The results of Tukey's HSD test are shown in Figure 5.11 and Figure 5.12.

The results presented in the left chart of Figure 5.11 indicate that, in the case of Dry Protocol-1, the levels of $CaCO_3$ precipitation in each round is statistically different regardless of the type of soil used.

On the other hand, the right chart in Figure 5.9 demonstrates that, irrespective of the number of rounds, the effect of soil type on $CaCO_3$ precipitation is not statistically different for soil type S1 and S3; but the effect of other combinations of soil types are statistically different.

The results in Figure 5.12 presents the interaction between soil type and rounds for Dry Protocol-1.

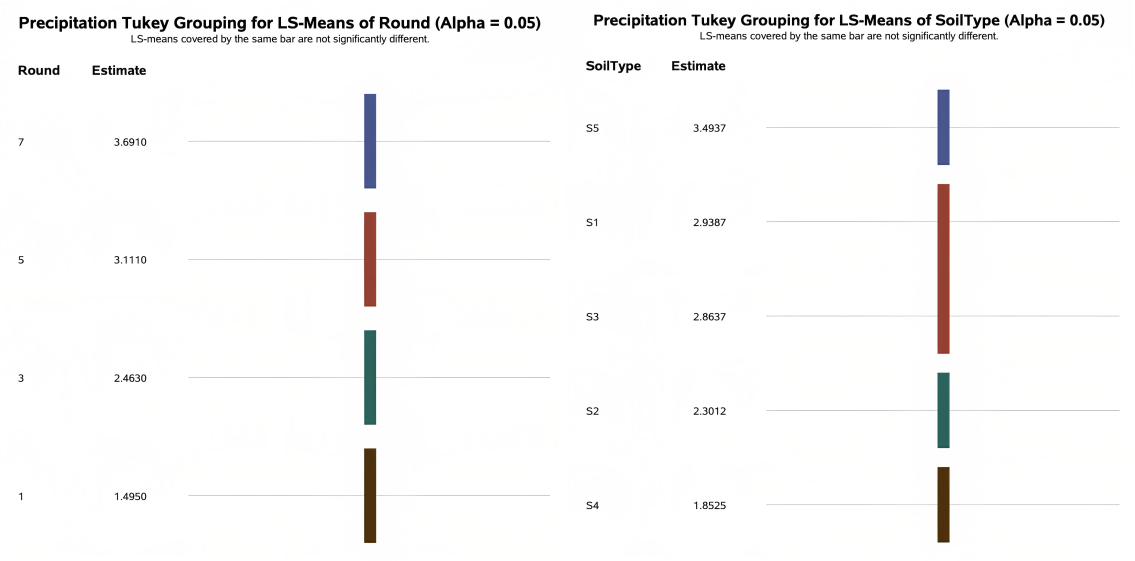

Figure 5.11: Tukey's HSD for Dry Protocol-1 Round (Left) and Soil Type (Right)

The top-left chart in Figure 5.12 shows that for Dry Protocol-1, for round 1, the effect of soil type S1, S3 and S5 on $CaCO_3$ precipitation are not statistically different and effect of each of S2 and S4 is different from rest of others.

The top-right chart in Figure 5.12 shows that for Dry Protocol-1, for round 3, the effect of soil type S1, S2 and S5 on $CaCO_3$ precipitation are not statistically different and effect of each of S4 and S5 is different from rest of others.

The bottom-left chart in Figure 5.12 shows that for Dry Protocol-1, for round 5, the effect of soil type S1, S2 and S4 are not statistically different; the effect of soil type S2 and S4 are not statistically different but effect of soil type S5 is statistically different from rest of others.

The bottom-right chart in Figure 5.12 shows that for Dry Protocol-1, round 7, the effect of soil type S1, S3, S4 and S5 are not significantly different from each other but the effect of soil type S2 is statistically different from rest of others.

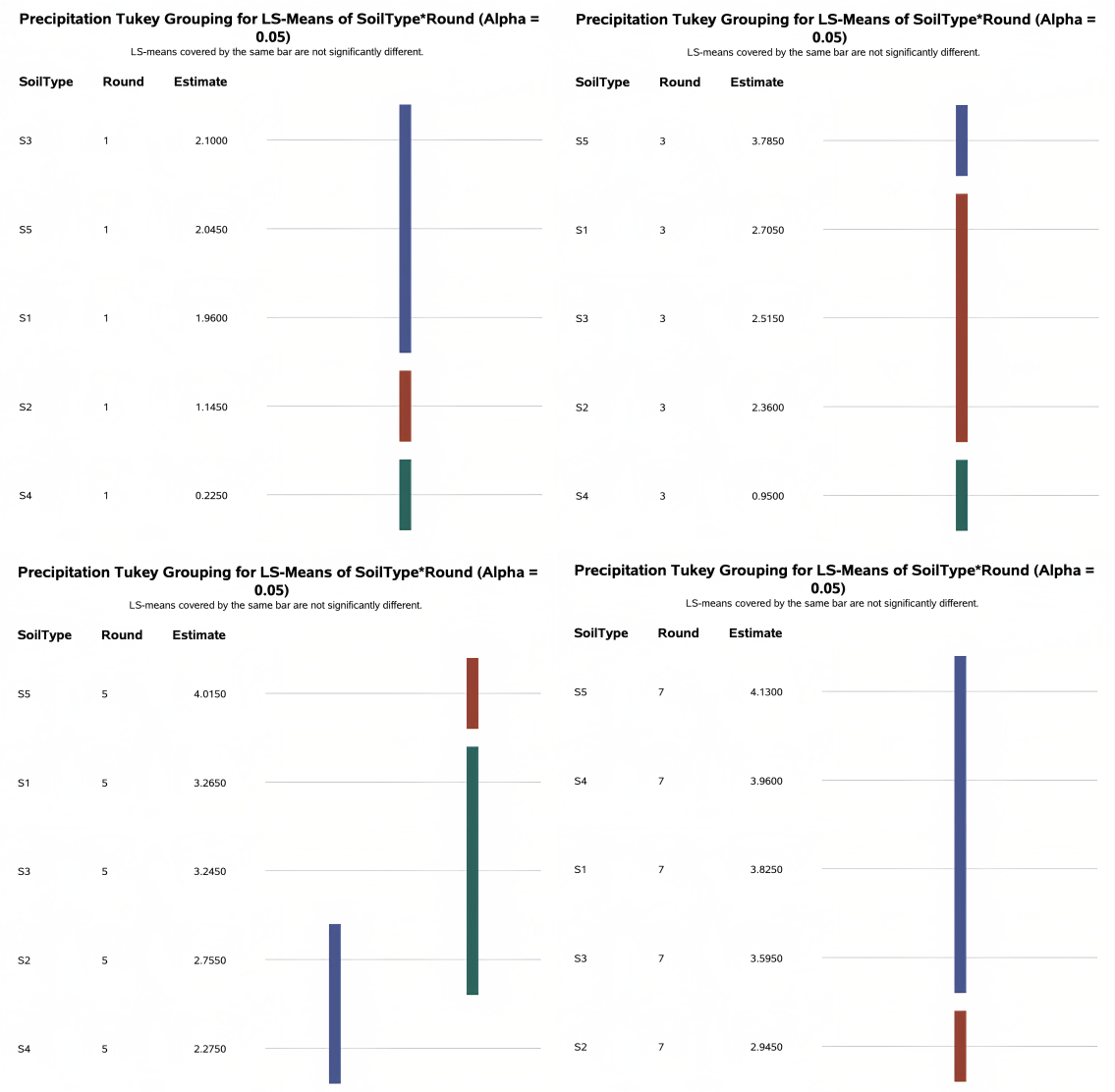

Figure 5.12: Tukey's HSD for Dry Protocol-1 Round and Soil Type Interaction

5.4.2.3 Two-Way ANOVA with Dry Protocol-2 as constant

We perform two-way ANOVA keeping Dry Protocol-2 as constant. We set the significance level (α) to 0.05. We get the source table as shown in Table 5.9.

Table 5.9: ANOVA Source Table for Dry Protocol-2 as constant

Effect	**Numerator Degree of Freedom**	**Denominator Degree of Freedom**	**F Values**	**P-value**
Soil Type	4	5	20.57	<0.0026
Round	3	15	9.41	0.0010
Soil Type $\times$*Round*	12	15	1.36	<0.2830

Conclusion: The p-value for the Soil Type is 0.0026 which is less than our significance level of α (0.05). Therefore, we reject our 1st null hypothesis and conclude that the effect of at least one of the soil types is different from rest of the others for Dry Protocol-2. The p-value for the Round is less than 0.0010 which is also less than our significance level of α (0.05). Therefore, we reject our 2nd null hypothesis and conclude that the effect of at least one of the rounds is different from rest of the others for Dry Protocol-2. The p-value for the interaction between Soil Type and Round is 0.2830, which is also above our significance level ($\alpha = 0.05$). Thus, we do not reject the null hypothesis and conclude that there is no significant interaction effect between Soil Type and Round of Treatments on $CaCO_3$ precipitation for Dry Protocol-2. This indicates that the combined effect of these factors does not differ from what would be anticipated based on their individual effects alone.

Since the null hypotheses I and II are rejected, we perform post-ANOVA analysis

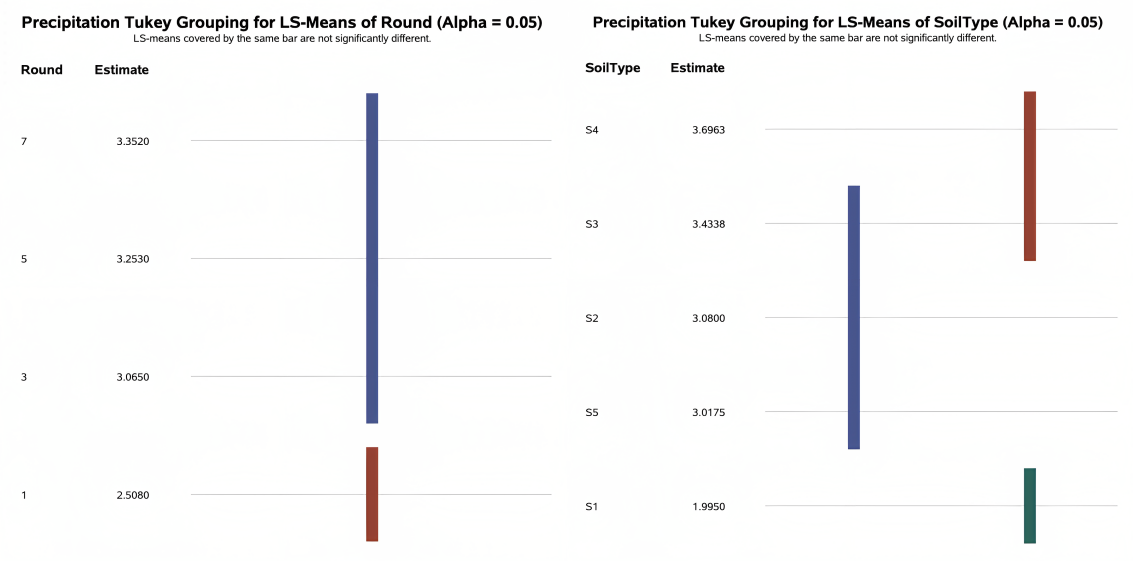

Figure 5.13: Tukey's HSD for Dry Protocol-2 Round (Left) and Soil Type (Right)

using Tukey's HSD to see the effect of which all soil type and rounds differ from each other. The results of Tukey's HSD test are shown in Figure 5.13.

The results presented in the left chart of Figure 5.13 indicate that, in the case of Dry Protocol-2, regardless of soil type, the effect of round 3, 5 and 7 on $CaCO_3$ precipitation are not statistically different but the effect of round 1 is statistically different from rest of others.

On the other hand, the right chart in Figure 5.13 demonstrates that, irrespective of the number of rounds, the effect of soil type on $CaCO_3$ precipitation is not statistically different for soil type S2, S3 and S5; the effect of soil type is not statistically different for soil type S3 and S4; but the effect of soil type S1 is statistically different from the rest of others.

5.4.2.4 Two-Way ANOVA with Dry Protocol-3 as constant

We perform two-way ANOVA keeping Dry Protocol-3 as constant. We set the significance level (α) to 0.05. We get the source table as shown in Table 5.10.

Table 5.10: ANOVA Source Table for Dry Protocol-3 as constant

Effect	**Numerator Degree of Freedom**	**Denominator Degree of Freedom**	**F Values**	**P-value**
Soil Type	4	5	21.77	$<$0.0023
Round	3	15	17.42	$<$0.0001
Soil Type $\times$*Round*	12	15	1.77	0.1483

Conclusion: The p-value for the Soil Type is 0.0023 which is less than our significance level of α (0.05). Therefore, we reject our 1st null hypothesis and conclude that the effect of at least one of the soil types is different from rest of the others for Dry Protocol-3. The p-value for the Round is less than 0.0001 which is also less than our significance level of α (0.05). Therefore, we reject our 2nd null hypothesis and conclude that the effect of at least one of the rounds is different from rest of the others for Dry Protocol-3. The p-value for the interaction between Soil Type and Round is 0.1483, which is also above our significance level ($\alpha = 0.05$). Thus, we do not reject the null hypothesis and conclude that there is no significant interaction effect between Soil Type and Round of Treatments on $CaCO_3$ precipitation for Dry Protocol-3. This

indicates that the combined effect of these factors does not differ from what would be anticipated based on their individual effects alone. Since the null hypotheses I and II are rejected, we perform post-ANOVA analysis using Tukey's HSD to see the effect of which all soil type and rounds differ from each other. The results of Tukey's HSD test are shown in Figure 5.14.

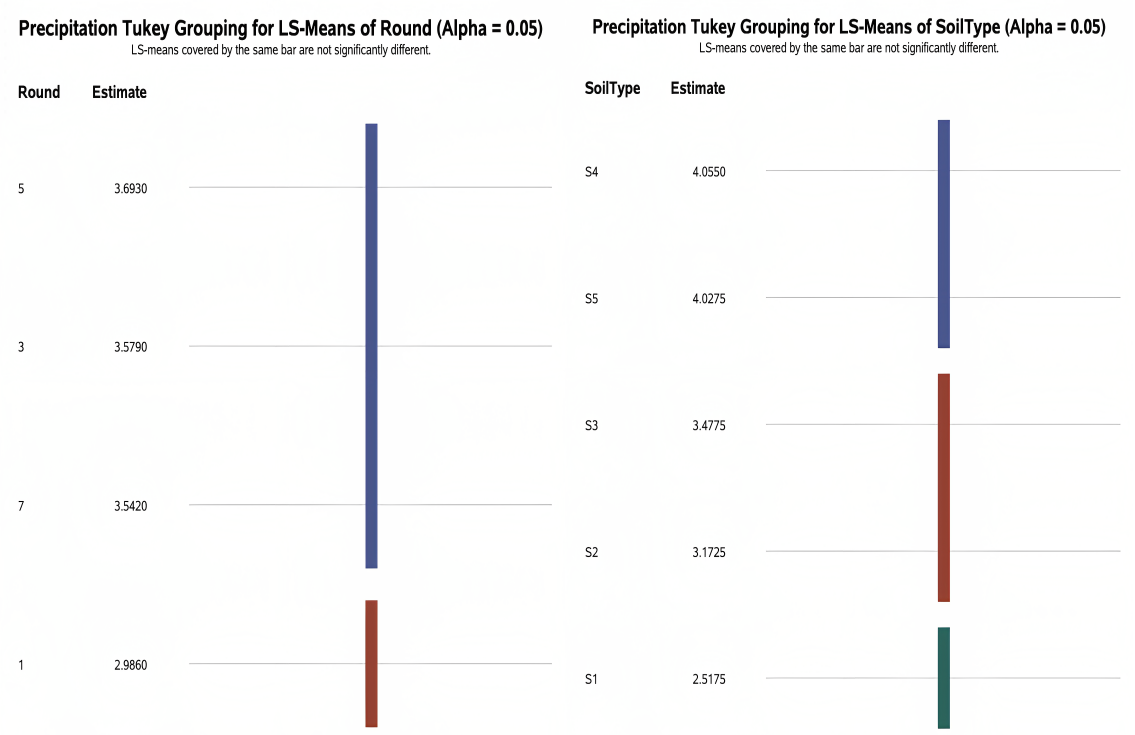

Figure 5.14: Tukey's HSD for Dry Protocol-3 Round (Left) and Soil Type (Right)

The results presented in the left chart of Figure 5.14 indicate that, in the case of Dry Protocol-3, regardless of soil type, the effect of round 3, 5 and 7 on $CaCO_3$ precipitation are not statistically different but the effect of round 1 is statistically different from rest of others.

On the other hand, the right chart in Figure 5.14 demonstrates that, irrespective of

the number of rounds, the effect of soil type on $CaCO_3$ precipitation is not statistically different for soil type S4 and S5; the effect of soil type is not statistically different for soil type S2 and S3; but the effect of soil type S1 is statistically different from the rest of others.

CHAPTER 6:

SUMMARY, FINDINGS AND RECOMMENDATIONS

The concluding chapter encompasses a concise overview of the research endeavors undertaken within the ambit of this study, alongside significant findings. Conclusions have been drawn based on the study's findings, allowing for insightful deductions. Furthermore, recommendations have been proposed for future research endeavors aimed at augmenting comprehension of the problem at hand and devising appropriate solutions.

6.1 Summary

The primary aim of this research endeavor was to develop dry mixing protocols that enable direct incorporation of compounds into soil, allowing water to be added subsequently, thus eliminating the need for preparing and mixing large quantities of treatment solutions. The performance of these dry protocols in treating soil using Microbially Induced Calcium Carbonate Precipitation (MICP) was evaluated, with the goal of generating sufficient calcium carbonate precipitate to enhance soil stabilization. A comparative analysis was conducted with the conventional method that has been employed for MICP application over several years.

The objective of this thesis was to introduce a novel application method for MICP

treatment that offers increased convenience, ease of implementation, and potential cost savings. To achieve this, three dry protocols, namely dry protocol-1, dry protocol-2, and dry protocol-3, were developed for MICP application through biostimulation. These protocols involved the mixing of dry forms of enrichment and cementation compounds into various soil types, including four naturally occurring soils (S1, S2, S3, S5) and one artificially mixed soil (S4), to explore alternative approaches. The experimental procedure consisted of an initial enrichment round followed by seven rounds of cementation treatment, each lasting 48 hours. The performance assessment involved monitoring the pH levels, calcium carbonate precipitation, and free swell index. Two soil samples were prepared for each soil type, and the MICP treatment was administered using the four protocols (three dry and one conventional). pH measurements were taken after each treatment round, while calcium carbonate precipitation was measured after the 1st, 3rd, 5th, and 7th rounds of treatment. Furthermore, the free swell index test was conducted for soils with a significant fines content (S4 and S5) after completing the treatment process.

6.2 Research Findings

The major findings obtained from this study are presented as follows:

1. The pH levels of the soils, which serve as an indicator of urease activity, were higher when treated with the conventional protocol and dry protocol-1. In contrast, the pH levels were relatively lower when using dry protocol-2 and dry protocol-3. As the treatment progressed, dry protocol-2 and dry protocol-3 led to a significant reduction in pH for the four soils, except for soil S5, where the pH remained relatively constant after two rounds of cementation using dry protocol-3.

2. Calcium carbonate precipitation exhibited a gradual increase after each round of cementation for both the conventional protocol and dry protocol-1. However, precipitation was initially high in the early treatment rounds for dry protocol-2 and dry protocol-3, but it subsequently decreased or showed only a slight increase as the treatment progressed.

3. Among the five soils examined, the conventional protocol resulted in the highest amount of calcium carbonate precipitation after seven rounds of cementation.

4. Dry protocol-3 demonstrated the highest calcium carbonate precipitation after the first round of cementation in all five soils. Dry protocol-2 produced the second-highest precipitation in four soils and ranked third in soil S5. Decreases in calcium carbonate precipitation were observed after a few rounds of cementation when using dry protocol-2 and dry protocol-3.

5. The significance of mixing in MICP was clearly apparent. Notably, dry protocol-3, which involved adding and mixing water for each treatment round, resulted in higher precipitation compared to dry protocol-2, where mixing was not performed, and water was only added for successive rounds of treatment.

6. The selection of protocols should consider the desired amount of precipitation and the available time for MICP, as different protocols yielded varying amounts of calcium carbonate precipitation during different rounds of cementation treatment.

7. There was a direct correlation between calcium carbonate precipitation and the free swell index. The sequence of effectiveness in reducing soil swelling was as

follows: conventional protocol, dry protocol-3, dry protocol-1, and dry protocol-2.

6.3 Recommendation for Future Research

This research serves as an initial investigation into the feasibility of Microbially Induced Calcium Carbonate Precipitation (MICP) in soils through the direct mixing of dry chemical compounds into the soil, followed by the addition of water without the need for treatment solutions. The collected data presented in this study provide support for the effectiveness of MICP in soils using various dry protocols. To further advance the understanding and application of dry mixing protocols for calcite precipitation in soils, several potential avenues for future research are outlined below:

1. Explore the bacterial activity within the soil as the treatment progresses through multiple rounds. Conducting urease activity tests on these soils would provide insights into their ability to hydrolyze urea, leading to ammonium release.

2. Perform strength tests on soil at different stages of treatment to gain a deeper understanding of the calcite precipitates' mechanical properties and strength characteristics.

3. Develop a comprehensive field study on a larger scale to validate the findings and assess the practical applicability of dry protocols as an alternative approach for implementing MICP in real-world soil scenarios.

4. Investigate the influential factors that contribute to calcite precipitation in dry protocols, aiming to identify key parameters that can optimize the effectiveness of the process.

5. Consider alternative methods to determine the realistic free swell index of biostimulated soils, as the current process of pulverization during the free swell test may potentially disrupt the calcite bonds. Exploring alternative approaches would provide a more accurate assessment of the swelling behavior of biostimulated soils.

By addressing these research recommendations, further advancements can be made in the understanding and practical implementation of dry mixing protocols for calcite precipitation in soils, ultimately enhancing the applicability and efficacy of MICP in soil stabilization efforts.

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