

REPUBLIC OF TÜRKİYE
YILDIZ TECHNICAL UNIVERSITY
GRADUATE SCHOOL OF SCIENCE AND ENGINEERING

COMPARATIVE ANALYSIS OF BACTERIAL HEALING
EFFICIENCY IN CEMENT-BASED MORTAR USING
CAVE BACTERIA AND *SPOROSARCINA PASTEURII*

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BACTERIA AND *SPOROSARCINA PASTEURII***

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This work is dedicated to the pillars of my life:

To my father, whose memory is forever etched in my heart, for instilling in me the value of knowledge.

To my mother, for her endless sacrifices and unconditional love.

To my wife, my partner in every endeavor, for her unwavering support and belief in me.

To my children, my pride and joy, for inspiring me to strive for excellence.

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LIST OF SYMBOLS

A6	<i>Viridibacillus arenosi</i>
ATCC	American Type Culture Collection medium
B4	Bacterial precipitation medium
C15	<i>Streptomyces spororaveus</i>
C, CB	Samples with and without bacteria exposed to flexural loads
D25	<i>Bacillus zhangzhouensis</i>
E31	<i>Stenotrophomonas rhizophila</i>
E34	<i>Serratia quinivorans</i>
F, FB	Samples with and without bacteria exposed to Freeze-thawing
Fc	compressive strength
Ft	Flexural strength
H4, H6, H8	Samples exposed to high temperatures (400, 600, 800 °C)
SP	<i>Sporosarcina pasteurii</i>
TSB	Tryptic Soy Broth medium

LIST OF ABBREVIATIONS

CWA	Capillary Water Absorption Test
DSMZ	German Collection of Microorganisms and Cell Cultures
EDS	Energy-dispersive X-ray spectroscopy
SEM	Scanning Electron Microscopy analysis
UPV	Ultrasonic pulse velocity
WA	Water Absorption Test
XRD	X-Ray diffraction analysis

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ABSTRACT

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This research aims to study the bacterial healing process of cement mortar samples exposed to durability effects using bacteria characterized by their ability to precipitate calcium carbonate. *Sporosarcina pasteurii* is widely used for bacterial healing. This study aims to compare *S. pasteurii* performance with five strains of bacteria isolated from caves (*Viridibacillus arenosi*, *Streptomyces spororaveus*, *Bacillus zhangzhouensis*, *Stenotrophomonas rhizophila* and *Serratia quinivorans*). Cement mortar samples were exposed to high temperatures and freeze-thaw effects to create microcracks. Microcracks were also performed by applying flexural strength loads. Then, the formed microcracks were healed using spraying and injection of the bacterial solution for the first group for 90 days. The control and healed samples were conducted using flexural and compressive strength, water absorption, capillary water absorption, and ultrasonic pulse velocity (UPV) tests.

Microstructural analysis was also conducted to evaluate the bacterial healing products. Subsequently, statistical analysis was performed using the results of these tests to compare the various bacteria efficiencies. As a result of the statistical analysis, the total efficiency scores obtained in the statistical analysis for *Stenotrophomonas rhizophila* were 119, 112 for *Viridibacillus arenosi*, and 105 for *S. pasteurii*. Thus, *Stenotrophomonas rhizophila* and *Viridibacillus arenosi* showed the best healing performance compared to the other bacteria strains.

Keywords: Self-healing mortar, microcrack repair, cave bacteria, healing efficiency, durability, mechanical properties



ÇİMENTO ESASLI HARÇTA MAĞARA BAKTERİLERİ VE *SPOROSARCINA PASTEURII* İLE BAKTERİYEL İYİLEŞTİRME ETKİNLİĞİNİN KARŞILAŞTIRMALI ANALİZİ

Issam ALİ

İnşaat Mühendisliği Anabilim Dalı
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Bu araştırma, kalsiyum karbonatı çökeltme yetenekleri ile karakterize edilen bakteriler kullanılarak dayanıklılık etkilerine maruz bırakılan çimento harcı örneklerinin bakteriyel iyileşme sürecini incelemeyi amaçlamaktadır. *Sporosarcina pasteurii*, bakteriyel iyileşme için yaygın olarak kullanılmaktadır. Bu çalışma, *S. pasteurii* performansını mağaralardan izole edilen beş bakteri suşu (*Viridibacillus arenosi*, *Streptomyces spororaveus*, *Bacillus zhangzhouensis*, *Stenotrophomonas rhizophila* ve *Serratia quinivorans*) ile karşılaştırmayı amaçlamaktadır. Çimento harcı örnekleri, mikro çatlaklar oluşturmak için yüksek sıcaklıklara ve donma-çözülme etkilerine maruz bırakıldı. Mikro çatlaklar ayrıca eğilme dayanımı yükleri uygulanarak gerçekleştirildi. Daha sonra, oluşan mikro çatlaklar, ilk grup için 90 gün boyunca bakteri solüsyonunun püskürtülmesi ve enjeksiyonu kullanılarak iyileştirildi. Kontrol ve iyileştirilmiş örnekler, eğilme ve basınç dayanımı, su

emilimi, kılcal su emilimi ve ultrasonik darbe hızı (UPV) testleri kullanılarak gerçekleştirildi. Bakteriyel iyileşme ürünlerini değerlendirmek için mikro yapısal analiz de yapıldı. Daha sonra, çeşitli bakteri etkinliklerini karşılaştırmak için bu testlerin sonuçları kullanılarak istatistiksel analiz gerçekleştirildi. İstatistiksel analiz sonucunda, *Stenotrophomonas rhizophila* için istatistiksel analizde elde edilen toplam etkinlik puanları 119, *Viridibacillus arenosi* için 112 ve *S. pasteurii* için 105 oldu. Bu nedenle, *Stenotrophomonas rhizophila* ve *Viridibacillus arenosi* diğer bakteri suşlarına kıyasla en iyi iyileşme performansını gösterdi.

Anahtar Kelimeler: Kendiliğinden iyileşen harç, mikro çatlak iyileştirme, mağara bakterileri, iyileştirme etkinliği, dayanıklılık, mekanik özellikler



1

INTRODUCTION

1.1 General

Self-healing concrete is a ground-breaking concept, particularly valuable for autonomic healing of existing structures. The primary objective of self-healing concrete is to restore the integrity of concrete by addressing discontinuities caused by cracking. Typically, two approaches are employed for self-healing cracks in concrete: the development of calcium carbonate and the use of mineral admixtures. The latter approach tends to be less effective as crack width increases, necessitating the exploration of alternative and innovative healing techniques [1], [2]. Among the new materials being explored for construction applications, bacteria have emerged as a promising option. Bacteria-based self-healing of cracks is gaining attention as an eco-friendly, clean, and green technique for the repair and maintenance of concrete structures [3]. This study focuses on the combination of filling materials and bacteria to enhance the self-healing capabilities of concrete. Improvement in the quality and durability of concrete is always a prime concern for researchers. In the field of sustainable construction, special emphasis has been placed on enhancing the durability of existing structures. Among various methods, autonomic self-healing has garnered significant interest from both academic and industrial communities [3]. This research work aligns with the same concept, aiming to achieve self-healing of concrete using bacterial species. Both sporing and nonsporing bacteria are investigated in this study. The bacterial cells of aerobic species, either in spore form or as vegetative cells, are encapsulated or not encapsulated in polyurethane. A comparative study on the properties and effectiveness of these bacterial species has been detailed, providing insights into their impact on the self-healing capabilities of concrete [1]. This study offers an in-depth analysis of using bacterial species, either free or encapsulated, and compares their efficiency in the self-healing process of bacterial concrete.

1.2 Literature Review

The ability to heal the cracks in the concrete both continuously and intermittently without any external effect is known as self-healing ability. Calcium carbonate precipitation caused by soluble carbon dioxide of calcium ion in concrete and its reaction leads to self-healing [4], [5]. Cement based naturally tends to heal itself, if such a healing is provided by bacteria, it is called autonomous healing. However, it is known that self-healing is effective for the superficial healing of cracks that are not greater than 300 μm formed by sedimentation [6], [7]. The cement has a high alkaline environment and therefore used bacteria should be able to live in this environment. It can produce endospores to be activated in cement matrix materials to survive in challenging conditions with reduced Nutrients [8]. There are studies on self-healing in concrete and mortar. 4-, 6- and 8-months water cured sample self-healing was studied [9]. The results of the experiments and studies show the ability of *Sporosarcina Pasteurii* and *Sposarcina* bacteria to produce useful substances in the process of self-healing, and these bacteria can live in very alkali environments. The pressure strength of mortar samples increased due to the accumulation of calcium carbonate in the pores. The silica gel and polyurethane showed that the permeability and durability improve concrete properties as a result of the precipitation of calcium carbonate by immobilized bacteria through self-healing of concrete by self-healing. Due to microbiologically derived calcite deposition technology in cement-based systems, permeability is reduced and rapid crack repair is achieved, and durability properties are observed to improve by reducing the penetration of harmful substances into the concrete [1]. The precipitation of microbiologically induced calcite is a biochemical process in which microorganisms stimulate the production of calcium carbonate (CaCO_3) [10]. In the literature, using this method, cracks caused by tension or bending up to 0.7 mm wide were eliminated and the water permeability of the mortar was reduced [11], [12].

1.2.1 Concrete Self-Healing Methods with Bacteria

1.2.1.1 Autogenous And Autonomous Self-Healing

Concrete is one of the construction materials that are structurally exposed to cracking. In areas where cracks occur, the cycle created by chemical, physical, and mechanical effects that lead to the closure of these cracks is called autogenous

healing. The autogenous healing process is caused by hydration and calcium carbonate precipitation. Autogenous healing is very effective [13]. Although autogenous healing can occur in all types of concrete, the levels and effectiveness of healing are variable. They cannot be fully determined since it depends on environmental and structural factors. Autogenous healing property was first observed in cement paste in 1956 [14]. Autogenous healing effectively closes cracks up to 300 microns wide and regains mechanical properties in cracks no larger than 10 microns. There are many studies and researches on autogenous healing in concrete, but there is no standard method to measure self-healing [6], [7]. The main reason for self-healing, which is expressed as the ability to heal cracks in the concrete element without the effect of any external factor, is due to the formation of calcium carbonate as a result of the interaction between calcium ions [4], [5]. If an additional substance is used to provide this self-healing, it is called autonomous healing. In his pioneering work on healing in cementitious systems, Dry (1994) proposed a self-healing mechanism involving intelligent chemical release from the fibers in the cement matrix [15]. In the following processes, this proposal was developed into a method of healing by biomineralization of the degraded and cracked cement matrix element due to internal and external effects. Biomineralization is a chain of biochemical reactions in which microorganisms trigger the formation of minerals [10]. The list of minerals to be obtained by these reactions is long, but the biomineralization product frequently used in cement-based materials is calcium carbonate (CaCO_3). Under suitable conditions, microorganisms can provide extracellular precipitation of calcium carbonate by various pathways including photosynthesis [16], ureolysis [17], denitrification [18], ammonification [19], sulfate reduction [20], and methane oxidation [21], depending on their metabolic properties.

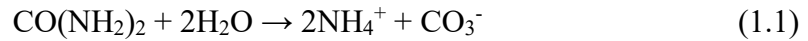
In order for the bacteria used in this system to be active in the cement matrix material, they must be able to live in a highly alkaline environment, perform oxygen respiration, and survive in difficult conditions and with little nutrition. Therefore, the use of endospore-forming bacteria is a great advantage [22]. Alkaliphilic bacteria can live in concrete in the form of endospores for hundreds of years. Over time, when the concrete structure is damaged, water penetrates into the cracks in it; bacteria consume oxygen and if Ca^{+2} is present in the environment, they form

CaCO₃ crystals and fill the cracks and voids [23], [24]. This increases the strength of the concrete [25], [26], [27]. It is also stated that water entry into the structural element is reduced by 25% compared to the control concrete by closing the voids [23].

The most commonly used bacteria in concrete so far are alkaliphilic strains that can break down urea in the environment with the urease enzyme. It has been stated in many studies that the urease enzyme in bacteria causes carbonate precipitation [28], [29], [30]. Urease activity is found in a wide range of microorganisms. However, some strains produce urease at particularly high levels. They increase the pH of the cement matrix environment by converting urea into ammonium and carbonate ions. Free Ca⁺² ions in the environment trigger the formation of CaCO₃. In more detail, the highly alkaline environment called the pore solution in concrete consists of dissolved alkali hydroxides such as K⁺, Na⁺, OH⁻ and small amounts of other ions such as Ca⁺², SO₄⁻². When the selected type of bacterial growth medium is released into the cement matrix, the bacteria are activated by the water entering through the cracks. Thus, they find the calcium source in the environment or provide it from externally added calcium chloride (CaCl₂) [31], calcium lactate (C₆H₁₀CaO₆) [3], [32], or calcium nitrates (Ca₂NO₃) [1], and take Ca⁺², SO₄⁻² into their body and convert it into limestone (CaCO₃).

The reactions that cause CaCO₃ precipitation with bacteria are shown in Equations 1 and 2. At this point, the amount of bacterial urease and its activity level are extremely important for this formation [33]

Bacterial urease



Bacterial groups that can break down organic components in alkaline environments and convert them into CO₂ and H₂O are also used in cement systems. CO₂ easily converts to CO₃⁻ in environments with high pH, and CaCO₃ precipitation occurs when Ca⁺² ion is present in the environment.

In studies conducted with *Bacillus cohnii*, *Bacillus pseudofirmus* and *Bacillus alkalinitrilicus* strains, CaCO₃ precipitation was triggered by oxidation of organic

components such as acetate and lactate and promising results were obtained [3], [12]. The reaction process is given in Equations 3, 4 and 5:

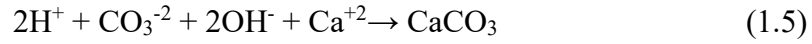
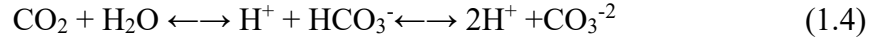
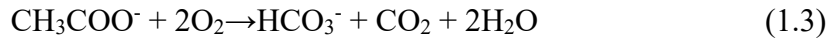


Table 1. 1 provides a comparison of some bacteria investigated to date in terms of urease activity and calcite formation capacity [33].

The *Bacillus* group, which produces urease enzyme and calcite, is the main organism used for multiple applications such as remediation of heavy metals and radionuclides, elimination of cracks in concrete and soil improvement [34], [35], [36], [37], *Bacillus megaterium* has been used to increase the strength and durability of concrete as a building material [31], [38], [39], [40]. The most commonly used bacteria in this category are *Bacillus sphaericus*, *Sporosarcina pasteurii* (also called *Bacillus pasteurii*), and *Bacillus megaterium*. Kashyap and Radhakrishna (2013) grew *Bacillus sphaericus* and *Sporosarcina pasteurii* bacteria in cultures of different densities, mixed them with cement paste and mortar, and poured them. In 7 and 28-day measurements, it was stated that concretes with *B. Sphaericus* and *S. pasteurii* bacteria increased the strength of concrete mortar by 50% and 28.2%, respectively, compared to traditional concrete, and calcite formation was observed in bacterial concrete in SEM and XRD analyses [41]. *S. pasteurii* is a non-pathogenic and endospore-producing bacterium that can tolerate extreme conditions and has an optimum pH of 9 for growth. Accordingly, many researchers have investigated this strain extensively for microbial cementitious systems (MICP) [28], [36], [42], [43], [44], [45], [46], [47], [48], Bang were the first to investigate the bacterial remediation mechanism in cementitious systems. They manually filled the artificially created cracks in mortar cubes (width = 3.175 mm, depth = 9.525 mm) with a mixture of *S. pasteurii* cells, nutrient medium components and sand.

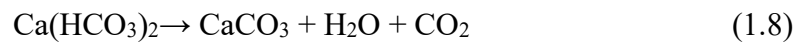
Table 1. 1 Urease producing bacteria from various sources and calcite and urease production amounts

Bacteria	Region of Isolates	Urease Activity U/ml	Calcite Precipitation	References
<i>Bacillus SP.</i> <i>CR2</i>	Mine Tailing Soil of Urumqi, Xinjiang, China	432	2.32 mg/cell	[49], [50]
<i>L. sphaericus</i> <i>CH5</i>	Mining Area of Gangwondo, Korea	—	980 mg/100 ml	[50]
<i>Sporosarcina</i> <i>Pasteurii</i>	Phenotypic Mutants, Institute of Microbial Technology, Chandigarh, India	550	—	[42]
<i>B. pasteurii</i> <i>NCIM 2477</i>	(NCIM) Pune, India	18	—	[35]
<i>K.</i> <i>Flava CR1</i>	Ore dust, Urumqi, China	472	—	[51]
<i>B.</i> <i>Megaterium</i> <i>SS3</i>	Calcareous Soils, Andhra Pradesh, India	690	187 mg/100 ml	[38], [51]
<i>B.</i> <i>Thuringiensis</i>	Calcareous Soils, Andhra Pradesh, India	620	167 mg/100 ml	[52]
<i>Halomonas</i> <i>sp. SR4</i>	Mine tailings, China	374.5	—	[53]

Then, the samples were immersed in urea- CaCl_2 medium for 28 days. The cracks of the samples were filled with biomixture and depending on the amount of bacteria, they showed an increase in compressive strength by 10-27% compared to controls without bacteria [23]. It was found that crack repair was successful only in surface cracks, and since the access of the organism to the necessary nutrient medium component and Ca^{+2} in the cracks remaining at the bottom was restricted and oxygen was reduced, there was no improvement in the cracks located at the bottom. The authors commented that the reason for this was that the first CaCO_3 crystals formed on the surface of the crack prevented the access of oxygen for the bacteria. When looking at the literature, it is seen that vegetative bacteria or spores are mixed into the concrete matrix with the water used in the preparation of cement paste in a certain ratio, including the necessary nutrients and CaCO_3 precursors [3], [54]. The generally suitable living temperature for bacteria is between $20-37^\circ$ [45], [54]. Although the bacteria are selected in accordance with the alkaline environment conditions of the concrete, it has been revealed by many studies that *Bacillus* strains that live at maximum $\text{pH} = 9$ levels and are added to the cement paste in vegetative form cannot maintain their viability for a long time in the $\text{pH} > 12$ environment of the concrete. In this case; studies have focused not only on the vegetative forms of the bacteria, but also on their endospore and encapsulated forms. When bacteria in the endospore form encounter unfavorable conditions, they minimize the cytoplasm surface, reduce their metabolic rate and take the spore form. However, even if they are used in the endospore form, the bacteria used lose their viability due to insufficient void diameter as a result of the filling of the voids with hydration products during the hydration of the cement. It is known that the size of bacterial spores is approximately $1.5-2\ \mu\text{m}$ and the pore diameters decrease to $1\ \mu\text{m}$ as a result of hydration [3]. In order to eliminate all these negativities affecting bacterial life, encapsulation of spores with various polymers, hydrogels, silica, zeolite, etc. has been resorted to [1], [26], [55]. The use of polyurethane is a frequently used material that is waterproof in bacterial encapsulation [43], [56], [57]. However, it is a disadvantage that polyurethane creates diffusion restrictions for bacteria to reach the environment they need [54]. The healing performance of cementitious materials is analyzed by chemical and mechanical tests or non-destructive test methods. Permeability and strength measurements of cementitious systems and observation of microstructure with optical microscopy are frequently preferred

methods. Granger observed autogenous healing by continuing to cure the samples that were loaded by creating a controlled pre-crack with the crack mouth opening displacement (CMOD) method during the flexural test [57]. Researchers preferred the resonance frequency method due to its ease of application to monitor autogenous healing [58]. As mentioned before, the most commonly used bacteria in autonomous healing are bacteria with positive urease activity. However, these bacteria can also cause concrete deterioration. Ammonium, which is produced as a byproduct of urea hydrolysis, can also act as a mild acid in alkaline environments. These bacteria react with hydroxide ions to form ammonia in the concrete; ammonia is oxidized to produce nitric acid, which reacts with the precipitated CaCO_3 to form a highly soluble component called calcium nitrite. The dissolution of this component can cause the concrete matrix to deteriorate [59]. For these reasons, bacteria with negative urease activity and capable of forming CaCO_3 precipitation through different pathways should not be ignored. In this context, cave bacteria have the potential to reduce these negativities.

Caves are underground cavities with openings to the surface and are divided into two as primary and secondary caves according to their formation. Caves formed at the same time as the main rock are primary caves, and lava tunnels can be given as examples of this group. Caves that contain rocks with soluble qualities such as limestone, gypsum, dolomite, sandstone or pebble in the formation environment and are shaped by groundwater are called secondary caves. Since the erosive effect of water on various rocks, creating underground cavities with different shapes and characteristics, is called karstification, secondary caves are also called karst caves. Chemically, the formation of caves is based on the loss of CO_2 by the dissolved carbon ions contained in the water that has reached the cave due to reasons such as heat, pressure decrease and evaporation, and the precipitation of CaCO_3 , forming cave structures such as stalactites and stalagmites called speleothems [60]. The chemical formation reactions of caves are given below.



It is known that in cave formations where chemical reaction types are also effective, biological reactions of the structures in the caves are also affected and thus take shape, and that many microorganisms play a role in the interactions that cause erosion and sedimentation on rocks [61]. The formation of cave structures and forms is not limited to chemical processes related to carbon dioxide alone. It has been observed that microorganisms found in many karst caves colonize calcite crystals and other mineral surfaces, and that microbial activities occur on and/or in carbon speleothems (stalactites, stalagmites, flowstones, cave pearls, etc.) [62]. Microorganisms that cause sediment formation directly contribute to the growth of speleothems and also determine the type of crystal materials [63]. Calcification microorganisms, characterized by complex crystal structures, create unique structures and environments due to their metabolic processes and products [64]. In this way, it is observed that structures such as stalactites/stalagmites maintain their continuous formation state depending on the activity of microorganisms [65], [66]. It is seen that studies on the calcium carbonate production abilities of microorganisms, especially in karst structures in the world, are intense. Baskar (2008) confirmed with experimental studies that microorganisms precipitate calcium minerals in the laboratory [67]. Ríos (2011) pointed out that the main factor determining the structure and formation of cave sediments is bacterial activity [68]. The fact that cave bacteria contribute to the formation of caves and cave structures by precipitating CaCO_3 also reveals that they may be more effective in the spontaneous improvement of concrete and mortar structures than bacteria isolated from other environments that precipitate CaCO_3 .

1.2.1.2 Self-Healing Methods and Techniques

The use of bacteria to treat concrete for self-healing purposes involves several innovative approaches. These methods exploit the ability of certain bacteria to precipitate calcium carbonate, which can fill cracks and pores in concrete. Healing techniques include three main methods for delivering bacteria to cracks or voids in concrete. The direct method involves exposing bacteria to damaged areas to stimulate self-healing, either by injection [43], [69], [70], [71], [72], [73], spraying [72], [73], [74], [75], [76], or immersion [72], [73], [77], [78], [79], [80]. The second method is the direct addition of bacteria to the structure of cementitious materials during mixing, either directly with the aqueous solution [81], [82], [83],

[84], or impregnated within one of the mixture elements [84], [85], [86], [87], [88]. The third method is the encapsulated addition method. Encapsulation refers to the inclusion of bacteria within the concrete matrix in the form of capsules. These capsules open when cracks form, which leads to the release of bacteria that activate the healing process [89], [90], [91], [92], [93]. Mahmoud Ziada, in his comparative study between the three techniques (injection, immersion, and spraying), showed that injection achieved the best results in terms of self-healing, followed by immersion and then spraying [94].

1.3 Objectives and Importance of the Thesis

This research aims to investigate in detail the biomineralization properties and self-healing mechanisms of cave bacteria that can replace *S. pasteurii* in the investigation of cracks that may occur in cement mortars.

The research objectives are summarized below:

- 1- Exposing cement mortar samples to durability effects, which are bending loads, high temperatures, and freeze-thaw cycles, in order to create micro-cracks and then perform the self-healing process on the samples.
- 2- The self-healing properties of cement mortar samples with cave bacteria will be determined and their performances in terms of self-healing will be compared with *S. pasteurii*.
- 3- Investigation of microstructural strengthening by applying a new product created by applying a medium injection method with or without bacteria on the cracks that will occur in the samples in order to heal the damage in the samples.
- 4- Investigation of self-healing of cement mortar samples with and without bacteria after durability effects.
- 5- Comparison of the healing capacities of cement mortars with cave isolates and *S. pasteurii*.
- 6- One of the most important objectives of the research is to try to determine the effect of bacteria isolated from caves of Türkiye on the self-healing potential of cement mortars with different metabolic properties and to compare their self-healing performance with *S. pasteurii*.

1.4 Hypothesis

This study aims to identify bacterial isolates from caves with different metabolic properties that could be an alternative for self-healing in concrete and mortar and to compare their performance with *S. Pasturii*, which is widely used in this field, in filling voids, repairing cracks, and improving mechanical properties.

Cracks occurring in cement-bound materials reduce the strength and durability of the material. This research aims to conduct a detailed study on the biomineralization property and self-healing mechanism of cave bacteria, which can be an alternative to *S. Pasturii*, for cracks that may occur in cement-bound concrete. For this reason, cement-bound samples are subjected to cracking effects upon loading, high temperature, or freeze-thaw cycles. The self-healing and independent recovery process of cave bacteria and *S. Pasturii* are carried out after cracks are made in cement-bound samples. The injection and spraying methods are used as the healing method. Samples contain bacteria in some samples and other samples are free of bacteria. Injection method is used for cracked samples by loading, while other samples subjected to high temperatures and freeze-thaw cycles are healed with bacteria spraying method. The mechanical properties, water permeability, and microstructural properties of healed and unhealed samples are determined and compared. The necessary information for the practical use of the injection and spray methods used in self-healing are provided. At the end of the project, the outputs are planned to be presented to the manufacturers. The mechanism to be developed within the scope of the proposed project will increase multidisciplinary research on this topic and accelerate practical studies in Türkiye, where the construction sector is very active.

2

MATERIALS AND METHODS

2.1 Materials

This study used Portland cement mortar samples in the prismatic form with dimensions of 40*40*160 mm. These samples were prepared according to the standards specification EN 196-1:2005 [95] . The mixture consisted of standard sand 1350 gr, Portland cement 450 gr (42.5 CEM II), 225 gr of water or bacteria inoculum, and 4.5 gr of fiber (1% of cement weight). The bacterial inoculum in mixes containing bacteria instead of water as shown in **Figure 2. 1**. Polypropylene fibers were added to the mixture at a rate of 1% in samples subjected to flexural loads and samples subjected to freezing-thawing to ensure control of artificial cracks. As for the samples exposed to high temperatures, basalt fibers were used at a rate of 1%. **Table 2. 1** shows the characteristics of basalt and polypropylene fibers.



Figure 2. 1 Materials used in the sample mixture

Table 2. 1 Characteristics of basalt and polypropylene fibers

Type of Fiber	Length (mm)	Diameter (mm)	Tensile Strength (MPa)	Specific Gravity	Modulus of Elasticity (GPa)
Basalt	24	0.02	4840	2.6-2.8	89
Polypropylene	12	0.018	1160	0.91	2-2.8

This study used six strains of bacteria, five of bacteria isolated from Dupnisa cave [96]. The accession numbers of these isolates are showed in **Table 2. 2**. In addition, *Sporosarcinia pasteurii* DSM33 brought from the German Collection of Microorganisms and Cell Cultures (DSMZ) was used as standard bacteria. In general, ureolytic bacteria are typically employed in biocement production due to their ability to induce calcium carbonate precipitation, this study investigated urease-negative strains to mitigate potential environmental toxicity associated with ammonia, a urease by-product. This approach aimed to develop a more sustainable bioconcrete application. **Figure 2. 2** shows the preliminary evaluation process to determine the healing performance by cave bacteria which confirmed that four selected bacterial strains (C15, D25, E31, and E34) lacked urease activity, demonstrating that precipitation can occur via alternative mechanisms. Calcium carbonate (CaCO_3) precipitation in B4 medium was observed on various days post-inoculation: specifically, A6 (day 4.), C15 (day 5.), D25 and E31 (day 1.), and E34 (day 3) [97]. The codes of the used bacteria are shown in **Table 2. 2**.

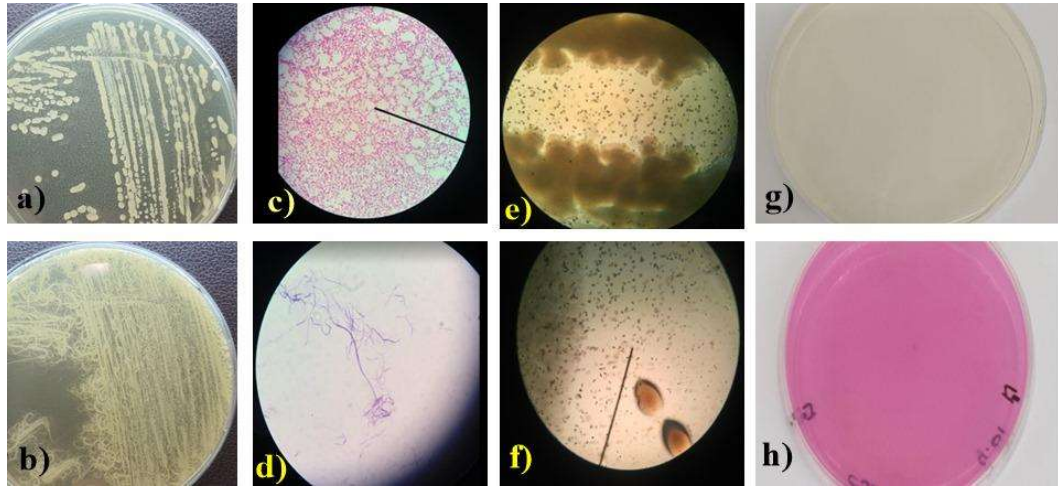


Figure 2. 2 Preliminary evaluation for the determination of healing performance by cave bacteria: (a-b) purification of isolates, (c-d) determination of gram staining characteristics of isolates (pink Gram negative; purple Gram positive), (e-f) CaCO_3 precipitation of isolates in B4 medium, and (g-h) Presence of urease enzyme in isolates (pink urease enzyme positive; yellow urease).

Table 2. 2 The codes of bacteria strains and accession numbers

Bacteria Strains	Code	Accession Number
<i>Sporosarcina pasteurii</i>	SP	MW405667
<i>Viridibacillus arenosi</i>	A6	MW405737
<i>Streptomyces spororaveus</i>	C15	MW405647
<i>Bacillus zhangzhouensis</i>	D25	MW405708
<i>Stenotrophomonas rhizophila</i>	E31	MW405679
<i>Serratia quinivorans</i>	E34	MW405667

2.2 Samples preparing

In this study, prismatic specimens were produced to test cement mortar in three main ways. First, C specimens without bacteria were used to test crack formation under flexural loads, while CB specimens with bacteria in the mixture. Second, H4, H6, and H8 specimens are exposed to 400, 600, and 800 °C temperatures. Third, F specimens without bacteria were exposed to freeze-thaw cycles, while FB specimens

with bacteria in their mixture. The bacterial inoculum used directly in the mix was prepared in the laboratory of the Department of Biology at Istanbul University. The bacterial solution consisted of 9×10^8 cells/ml (i.e., 3.0 according to the MacFarland standards) of each isolate suspended in the respective growth medium.

The samples were produced according to EN 196-1:2005 [95], Polypropylene or basalt fibers were added to the mixes at a proportion of 1% relative to the cement weight. The water in the mix was replaced by a bacteria inoculum at the same water ratio. The focus was on the mechanism of pouring the cement mortar into the molds, according to Annex A, vibrating table B [95], to ensure the homogeneity of the samples. Trial mixes were also made to verify this purpose. After demolding the prisms was coded, they were cured in water for 28 days at 20 °C as shown in . Then, the water absorption, flexural strength, compressive strength, and UPV tests were conducted on the samples. After demolding the prisms, the samples were coded. The cracked samples by flexural loads coded with C in samples without bacteria and CB in samples with bacteria. Samples subjected to 400 °C were coded H4, 600 °C H6, and 800 °C H8. Samples subjected to freeze-thaw were coded F for samples without bacteria and FB for samples with bacteria.



Figure 2. 3 Fresh samples in molds and cured samples in water

2.3 Inducing cracks in the samples

The method of cracking by controlling the crack width is of great importance in research to determine the efficiency of bacteria in healing. Researchers have used several mechanisms to create cracks [98]. In this study, cracks were made in C and CB samples using the Instron flexural strength machine, characterized by high loading speed flexibility. A loading speed of 0.2 kN/min was used to control the crack width to not exceed 300 μm , as shown in **Figure 2. 4**.



Figure 2. 4 Inducing crack in C and CB samples

Exposure of concrete or cement mortar samples to high temperatures (400, 600, and 800 °C) causes several physical and chemical changes that affect the properties and durability of the material. Rapid temperature changes lead to thermal stresses within the sample, which may cause cracking and, consequently, a decrease in compressive and flexural strength due to the decomposition of the cement structure and loss of chemically bound water. Samples at 28 days of age were heated in a high-temperature oven at a rate of 5 degrees/minute until the desired temperature was

reached as shown in **Figure 2. 5**. The samples were left for an additional hour at (400, 600, and 800 °C) and then went to cool [94].



Figure 2. 5 Inducing cracks in H4, H6, and H8 samples by exposing to high temperature

When concrete or cement mortar samples are subjected to freeze-thaw cycles, the frozen water inside the pores and cracks causes the volume to increase, generating internal stresses that may cause cracks to form. These cycles increase permeability, decreased strength and durability, and surface peeling. The effect of bacteria in the healing of samples containing bacteria can be studied directly or healed after exposure to freeze-thaw cycles [72]. This study produced seven group of samples: one group of control samples without bacteria and six groups containing a strain of bacteria added during mixing. The freezing and thawing process was carried out in light of ASTM - C666/C666M-03 (2008) [99], with some modifications in the procedures according to the nature of the study. Each group with the samples at 28 days was placed in a separate container according to the bacteria strains, and the samples were immersed in water as shown in **Figure 2. 6 (a, b)**. The samples were subjected to 300 freeze-thaw cycles. The duration of each cycle was 12 hours (6

hours freezing and 6 hours thawing). The temperature changed from -20 °C to +20 °C using the freeze and thaw cabinet as shown in **Figure 2. 6 (c)**.

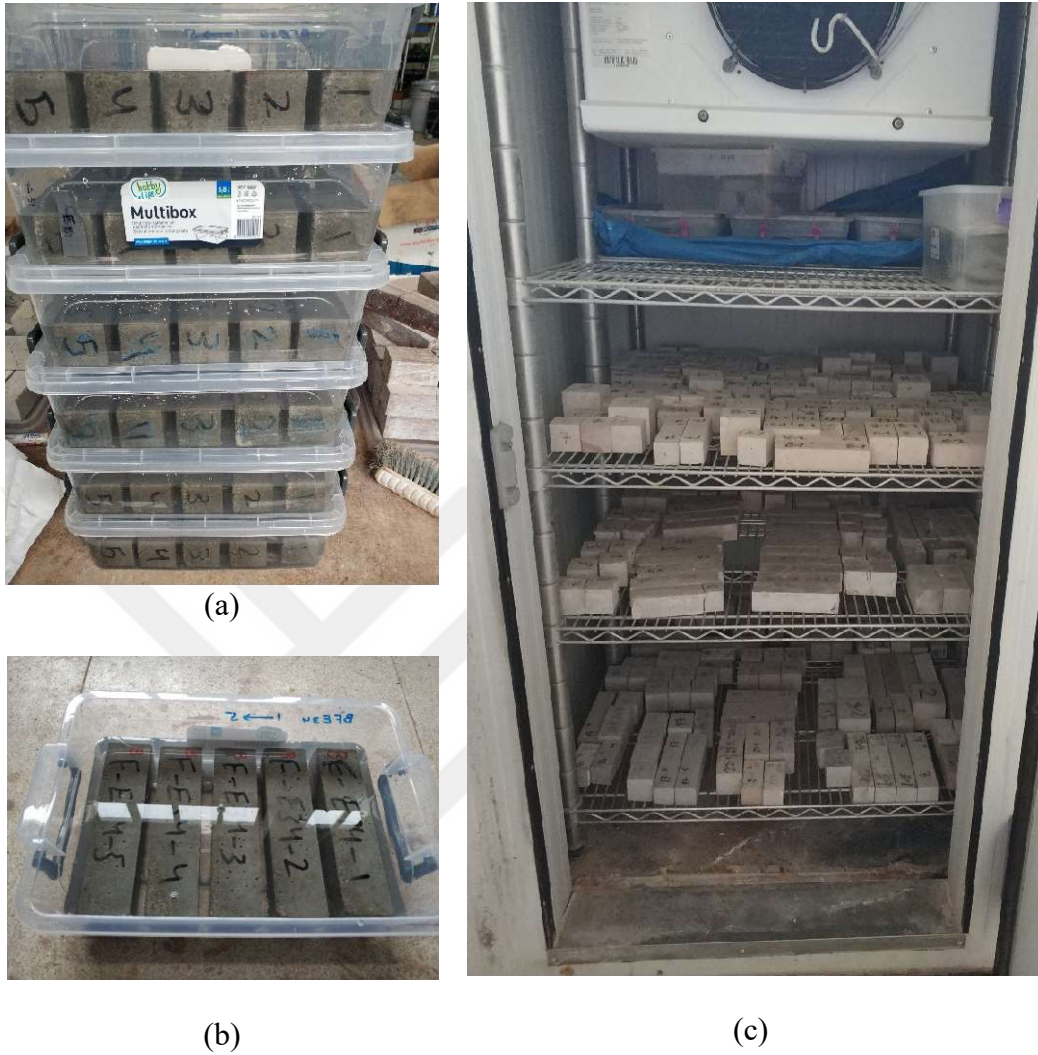


Figure 2. 6 F and BF samples exposed to freeze-thaw cycles, (a) sperate container for each bacteria strain, (b) immersion samples in water, (c) freeze-thaw cabinet

2.4 Bacterial healing procedure

Bacterial healing was carried out for C and CB samples for 90 days. The development of crack filling was monitored over time. Six bacterial strains were used in the healing process, with at least three samples per strain for each experiment. In addition, negative controls were established by using sterile media including specific medium of *Sprosarcinia pasteurii* (ATCC 1376: 20.0g Yeast extract; 10.0g (NH₄)₂SO₄; 0.13 M Tris buffer (pH 9.0)) and Tryptic Soy Broth used for the culture of other bacterial isolates. The injected solution and the bacteria were supplied with the medium weekly by the Department of Biology, Istanbul University

laboratory. Separate sterile bottles containing calcium lactate were also provided. At the beginning of each process, a quantity of the solution for injection (100 ml) was prepared by adding 10% volume (10 ml) of calcium lactate to the bacterial medium immediately before injection. The bacterial solution was injected into the cracks of the samples (200-300 μm width) every 12 hours using a syringe as shown in **Figure 2. 7**. The samples were stored separately by bacterial strain in plastic containers with lids throughout the healing period to ensure humidity and prevent bacterial contamination between bacterial strains. For samples exposed to high temperatures, disposable sprayers were used to apply the bacteria solution to the surface of the samples every 12 hours due to the nature of the cracks. The samples were stored separately by bacterial strain in plastic containers **Figure 2. 8**. Samples in groups F and FB were not further processed. Still, they were placed in a freeze-thaw cabinet to compare samples without bacteria with those containing bacteria and to compare bacterial strains.



Figure 2. 7 (a) Healing the samples by injecting the bacteria, (b) Storing samples in special containers for each strain of bacteria



(a)



(b)

Figure 2. 8 (a) Healing H4, H6, and H8 samples with spraying bacteria. (b) Used disposable sprayer

2.5 Methodology

This study aims to identify bacterial isolates from caves with different metabolic properties that could be an alternative for bacterial healing in mortar and to compare their performance with *S. Pasturii*, which is widely used in this field, in filling voids, repairing cracks, and improving mechanical properties. In this context, bacteria were used in the healing process on samples subjected to cracking by flexural loads, high temperatures, and freeze-thaw cycles. These samples were tested in several ways before and after the healing process to know the samples' characteristics and the bacteria's effectiveness in this process. The tests on the samples include flexural strength (Ft), compressive strength (Fc), water absorption (WA), capillary water absorption (CWA), and UPV. The results of all these tests were arranged and statistically analyzed to determine the most effective strains that could be alternatives to SP bacteria. **Table 2. 3** shows the tests applied to each group of samples.

Table 2. 3 Tests applied to each series of sample

Samples series	Ft	Fc	WA	CWA	UPV
Trial mix	✓	✓	✓		✓
C	✓				✓
CB	✓				✓
H4, H6, and H8	✓	✓	✓	✓	✓
F and FB	✓	✓	✓	✓	✓

2.5.1 Water absorption test

The water absorption test was performed according to ASTM C642 [54], as shown in **Figure 2. 9**, by calculating the samples' dry weights and saturated weights after heat exposure and bacterial healing. The samples (H4, H6, H8) were heated in an oven at 105 °C, then immersed for two days, and the saturated weight and the amount of water absorbed before and after healing were calculated. The dry weights of the FB samples to which bacteria were added during mixing were calculated by heating at 45 °C for 72 hours. The 28-day age samples were subjected to 300 freeze-thaw cycles for 12 hours each. The temperature changed from -20 °C to +20 °C.

Figure 2. 9 Weight of samples in water absorption test



2.5.2 Capillary water absorption test

According to (ASTM C1585-13) [100], the capillary water absorption test was performed on the samples after samples were exposed to temperatures (400, 600, and 800 °C), and the test was repeated on the same samples after healing with bacteria by spraying for 90 days. The dry weight of the sample was measured. The prisms were placed vertically in water with 5 mm of the sample submerged. The weights of the samples were measured at 1, 5, 10, 30, 60, 120, 180, 300, and 1440 minutes as shown in **Figure 2. 10**. The cumulative total of water absorbed by the samples was calculated.



Figure 2. 10 (a) CWA test, (b) Measurement of the rise of water in the sample

2.5.3 UPV test

UPV testing was performed according to BS EN 12504-4:2004 on samples before and after bacterial healing [101], as shown in **Figure 2. 11**. UPV test was conducted on the samples at 28 days of age and after exposing them to a temperature of (400, 600, and 800 °C). The test was repeated on the same samples after healing with bacteria by spraying for 90 days.



Figure 2. 11 UPV test

2.5.4 Flexural and compressive strength

The samples were tested for flexural and compressive strength according to the standards specification EN 196-1:2005 [95], as shown in **Figure 2. 12**. The study process is presented in the **Figure 2. 13**. In addition, scanning electron microscope (SEM), Energy dispersive spectroscopy (EDS), and X-ray diffraction spectroscopy (XRD) analysis were conducted as microstructural analysis to evaluate the healing performance of the samples.



Figure 2. 12 Flexural and compressive strength test

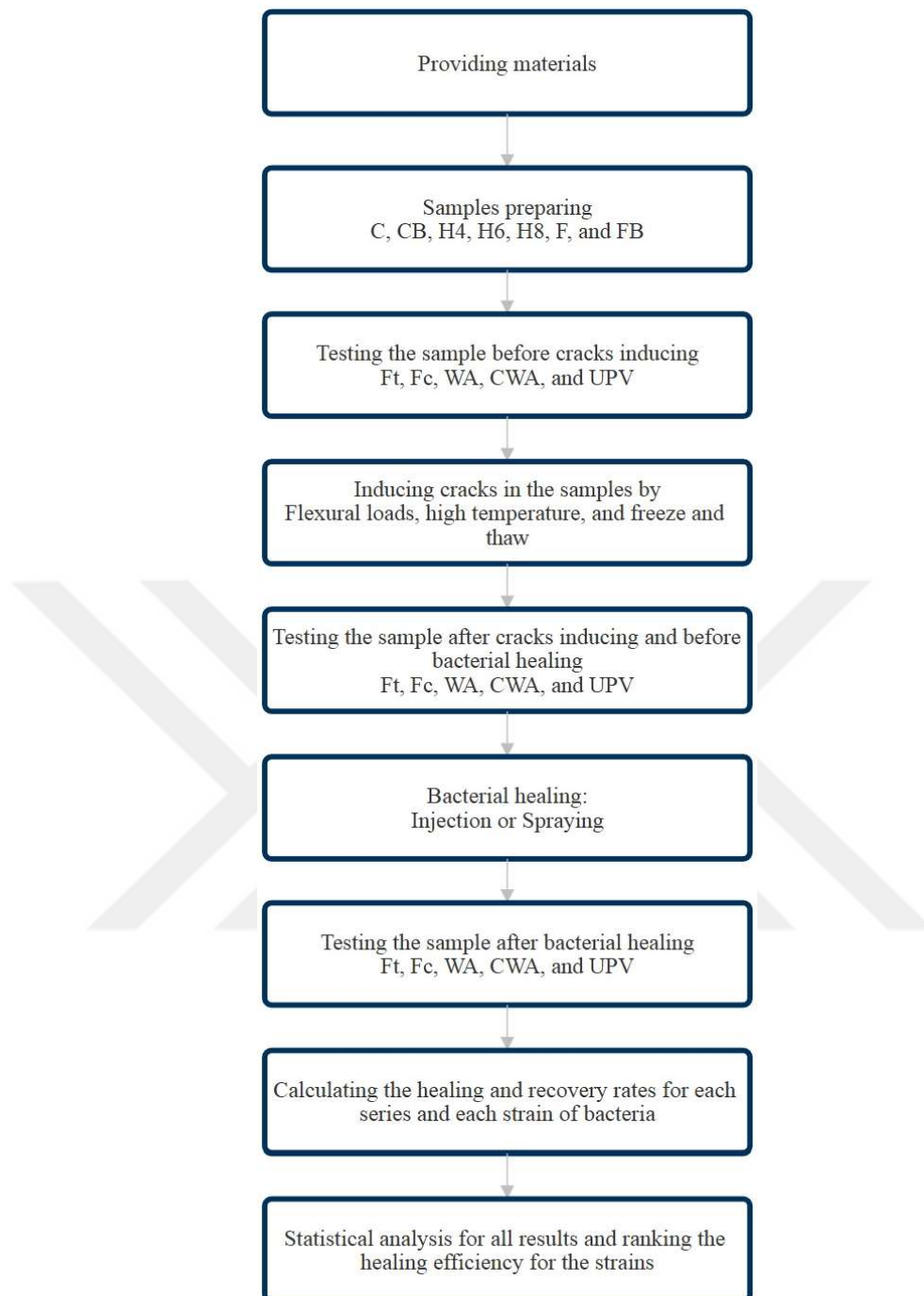


Figure 2. 13 Process flow chart

3

RESULTS AND DISCUSSIONS

In this study, tests were conducted on samples before and after cracking and after treating the samples with bacteria. During the 90-day bacterial treatment, the samples were monitored according to the types of bacteria with respect to the development of crack healing and its appearance. Tests (WA, CWA, Ft, Fc, and UPV) were performed on each set of specimens, before cracking, after exposure to durability effects, and after treating the specimens with bacteria. The results of these tests were compared for each stage to calculate the percentage of healing resulting from the bacteria used, and the results for each experiment were as follows:

3.1 Observable effects of bacterial healing of cracks

The samples were healed with bacteria for about 90 days until the cracks were almost filled. It was noted that the bacteria gave differences in filling rate and appearance. The strain C15 showed a faster filling speed for the cracks that were healed with injections than the other strains and the appearance of the bacteria in the areas where the injections were done tended to be yellow or brown. While the bacteria A6 and SP were white and the filling rate was relatively slow. In general, samples C and CB with flexural cracks of width ranging from 150 to 300 μm , the effectiveness of bacterial healing is lower and the duration is longer the larger the crack width regardless of the bacterial strain. **Figure 3. 1** shows cracks before and after bacterial healing in some samples from groups C and CB. The samples exposed to high temperature were healed by spraying method due to the nature of micro-cracks and the difficulty of implementing the injection method. In general, no visible cracks appeared on the samples exposed to 400 °C, while cracks appeared in the samples exposed to 600 °C, and to a greater extent in the samples exposed to 800 °C, as shown in the **Figure 3. 2**.

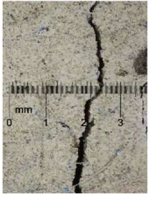
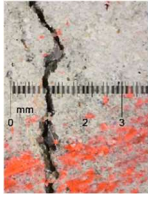
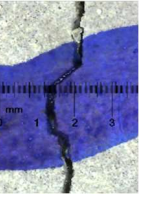
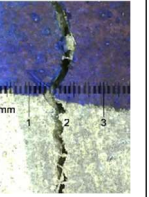
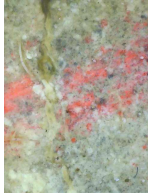
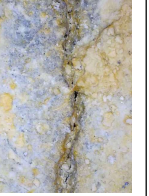
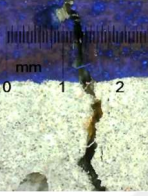
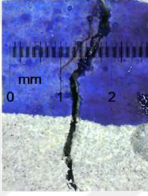
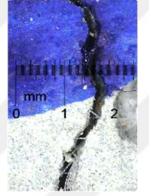
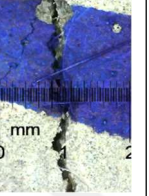
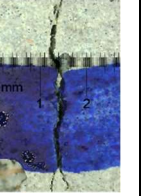
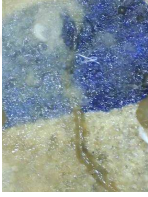
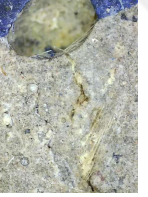
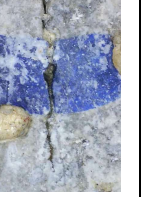
Seri	C-SP	C-A6	C-C15	C-D25	C-E31	C-E34
a						
b						
Seri	CB-SP	CB-A6	CB-C15	CB-D25	CB-E31	CB-E34
a						
b						

Figure 3. 1 C and CB samples in which cracks were made by flexural loads (a) before and (b) after bacterial healing.

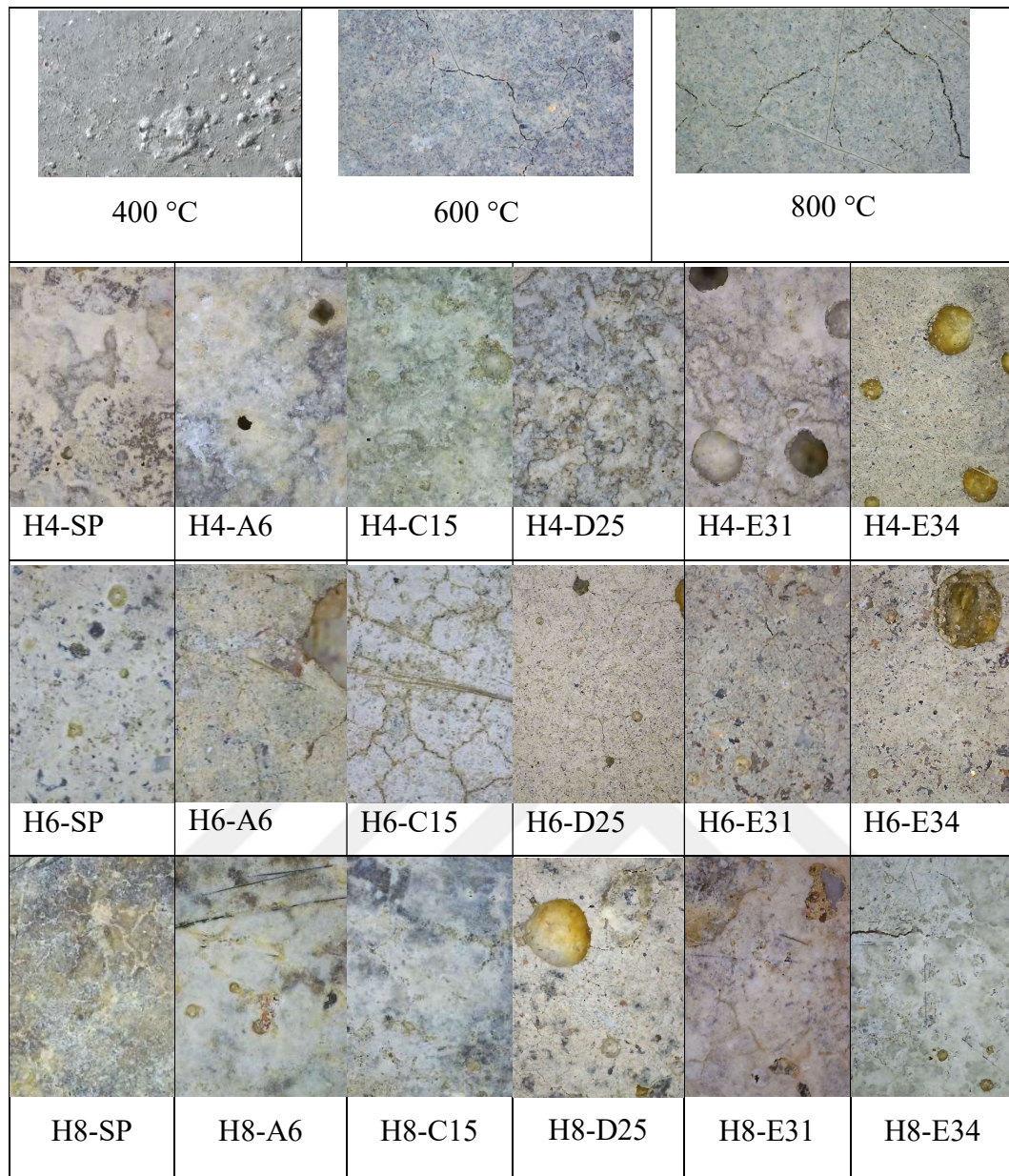


Figure 3. 2 Samples exposed to high temperature before and after bacterial healing

3.2 Water Absorption Test

The decrease in the water absorbed after healing compared to before healing, representing void reduction, was considered the percentage of recovery in the samples. It is known that exposing concrete to high temperatures increases the porosity of the concrete and the water absorption rate [102], [103]. Similarly, in this study, pores and cracks were formed in the specimens subjected to elevated temperatures due to water evaporation and dehydration. The results shown in **Table 3. 1** and **Figure 3. 3** confirmed that with increasing temperature, porosity, and water

absorption rate increase. The water absorption rate increased from (8-10%) in H4 samples to (11-12%) in H6 samples and to (12-15%) in H8 samples.

Table 3. 1 Water absorption test for the sample exposed to high temperature

Seri	Samples weight g			Void volume cm3			Healing percentatge
	At 28	At 400 °C, 600 °C, 800 °C	After Healing	V1	V2	ΔV	
H4-SP	566.28	508.525	561.20	57.76	52.68	5.08	8.80%
H4-A6	566.12	511.65	556.23	54.47	44.58	9.88	18.15%
H4-C15	567.42	516.85	559.07	50.57	42.22	8.35	16.51%
H4-D25	566.77	519.5	565.20	47.27	45.70	1.57	3.31%
H4-E31	563.80	516.55	559.78	47.25	43.23	4.02	8.51%
H4-E34	568.53	513.625	564.30	54.91	50.68	4.23	7.71%
ATTCC4	564.95	513.6	561.60	51.35	48.00	3.35	6.52%
TSB4	566.00	514	562.60	52.00	48.60	3.40	6.54%
H6-SP	567.38	502.4	565.13	64.98	62.73	2.25	3.46%
H6-A6	561.50	495.525	542.60	65.98	47.08	18.90	28.65%
H6-C15	561.20	494.075	550.93	67.13	56.86	10.27	15.29%
H6-D25	567.52	502.175	553.47	65.34	51.29	14.05	21.50%
H6-E31	563.83	498.975	546.70	64.86	47.72	17.13	26.42%
H6-E34	571.04	505.05	556.27	65.99	51.22	14.77	22.39%
ATCC6	576.85	501.35	552.25	75.50	50.90	24.60	32.58%
TSB6	561.00	496.05	560.90	64.95	64.85	0.10	0.15%
H8-SP	565.30	482.2	561.43	83.10	79.23	3.87	4.65%
H8-A6	565.77	488.1	557.47	77.67	69.37	8.30	10.69%
H8-C15	562.08	481.7	555.07	80.38	73.37	7.02	8.73%
H8-D25	562.20	487.5	560.90	74.70	73.40	1.30	1.74%
H8-E31	559.20	485.8	554.87	73.40	69.07	4.33	5.90%
H8-E34	568.68	495	568.37	73.68	73.37	0.32	0.43%
ATCC8	566.25	504.8	562.85	61.45	58.05	3.40	5.53%
TSB8	555.10	504.8	552.35	50.30	47.55	2.75	5.47%

In general, after the healing process, the void ratios of the healed samples were reduced. In the healed samples exposed to a temperature of 400 °C, the rate of void reduction ranged between 3.31% for samples with D25 bacteria and 18.1% for A6 bacteria. In samples exposed to a temperature of 600 °C, the percentages of void reduction ranged between 3.45 % for SP and 32.58% for the ATCC 1376 medium. In the samples exposed to a temperature of 800 °C, the percentage of void reduction ranged between 0.43% for E34 bacteria and 10.7% for C15 bacteria. This reduction occurred because the CaCO₃ precipitates filled the pores and cracks of the healed

samples. Studies indicated that bacteria healing enhanced water absorption rates significantly [72], [104]. **Figure 3. 3** shows that the effectiveness of bacteria in the healing process and filling the pores decreases. It was concluded that the efficiency of bacteria is greater in H6 samples than in H8 samples. This is because the size and dimensions of the voids in H8 samples are much larger, which reduces the efficiency of bacteria in filling these voids in terms of time and size. The maximum width of cracks caused by high temperature was 50 μm for H8 samples and 20 μm for H6 samples. No visible cracks were formed in the H4 samples. Earlier studies had shown that cracks less than 400 μm in width resulted in better crack closure [7], [72]. Also, since the healing was done by spraying, the bacteria's access to the deep pores is reduced in H8 samples.

Freeze and thaw cycles cause an increase in the pores in the concrete structure and thus increase its ability to absorb water [105]. The results show the percentage of void reduction ranged between (-16%) for D25 samples and 44% for SP samples, as shown in **Figure 3. 4**. The results show a decrease in the percentage of pores in all samples except sample D25, confirming the role of bacteria in precipitating calcium carbonate and closing the pores, especially when added during mixing, which ensures the distribution of the bacteria in the sample material homogeneously [27], [72]. It was also shown that SP and E31 bacteria strains have the best performance.

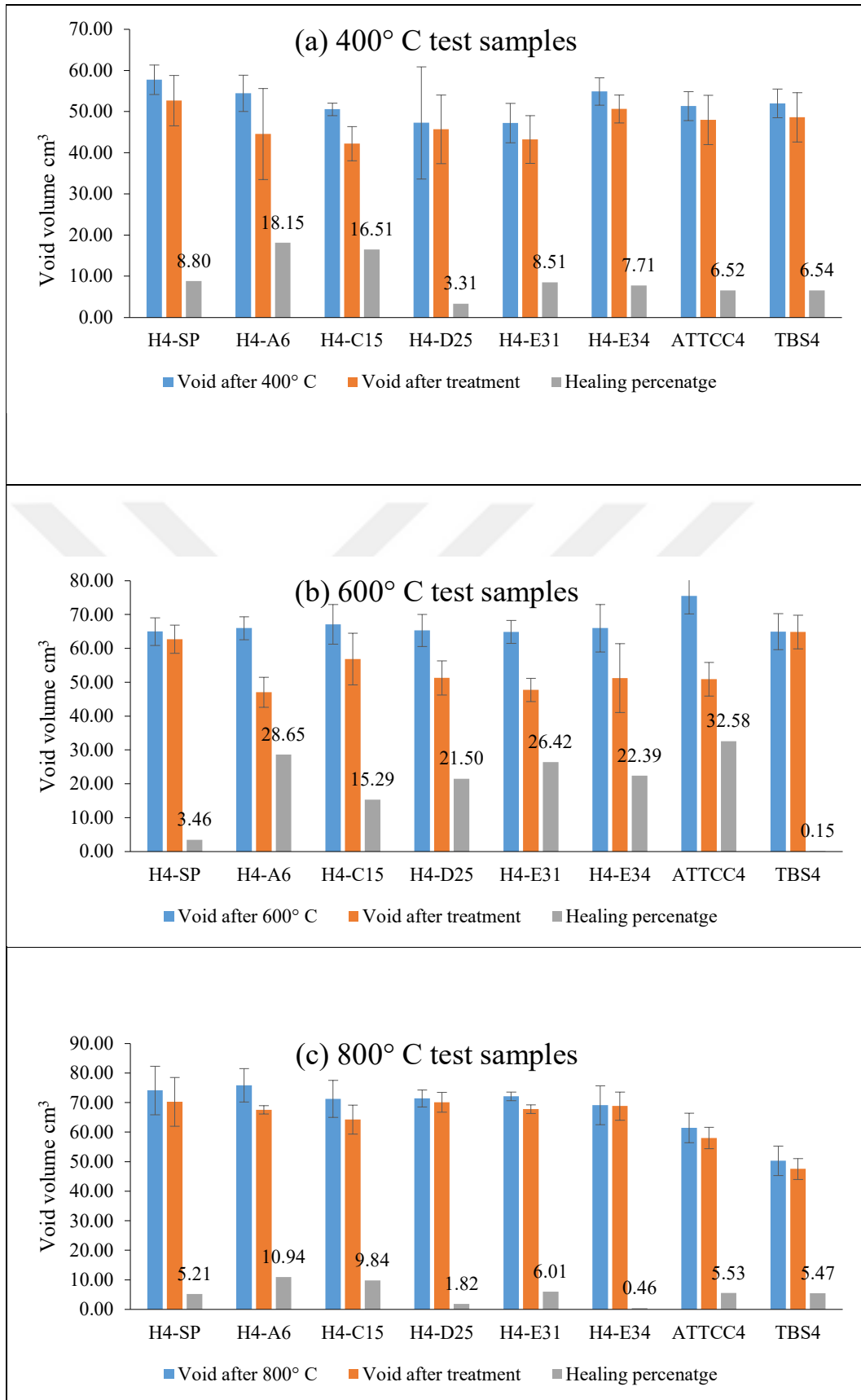
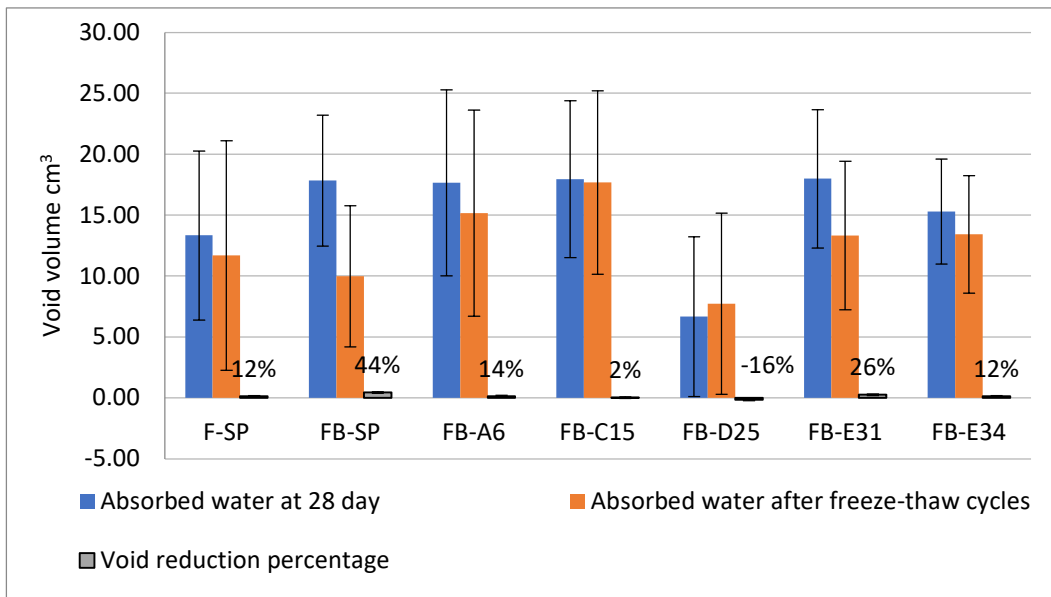


Figure 3. 3 Water absorption test results for samples exposed to high-temperature

Table 3. 2 Water absorption test results for samples exposed to freeze-thaw

Seri	Samples weight g				Void volume cm ³			Void Reduction Percentage
	At 28 days	After drying 45 °C	After freeze-thaw	After dry 45 °C	v1	v2	ΔV	
F-SP	562.68	549.4	542.13	530.44	13.33	11.69	1.64	12%
FB-SP	561.03	543.2	550.68	540.70	17.83	9.98	7.85	44%
FB-A6	564.65	547.0	552.60	537.43	17.65	15.17	2.48	14%
FB-C15	562.85	544.9	550.63	532.95	17.95	17.68	0.27	2%
FB-D25	568.47	561.8	558.70	550.97	6.67	7.73	-1.07	-16%
FB-E31	561.68	543.7	555.66	542.33	17.98	13.33	4.66	26%
FB-E34	557.80	542.5	546.92	533.50	15.30	13.42	1.88	12%

**Figure 3. 4** Water absorption test results for samples exposed to freeze-thaw

3.3 Capillary Water Absorption Test

Capillary water absorption is affected by porosity, the shape of the pores, their distribution, and the connectivity between the pores. The pores are increased by exposure to high temperatures, especially in the surface parts [103], [106]. In general, all samples' water absorption decreased within 24 hours. The percentage of decrease in water absorption ranged from 65% for E31 bacteria to 80% for SP bacteria in the samples exposed to a temperature of 400 °C, as shown in **Figure 3. 5**. The percentage of the decreases ranged from 68% for SP bacteria to 82% for A6 bacteria in the samples exposed to a temperature of 600 °C, as shown in **Figure 3. 6**. The decrease percentage ranged from 84% for SP bacteria to 88% for A6 bacteria in the samples exposed to a temperature of 800 °C, as shown in **Figure 3. 7**. The

use of bacteria has an apparent effect in healing the cracks of mortar and reducing capillary water absorption [80]. The reduction is caused by the bacterial products filling the cracks and pores [107]. The results show a decrease in the percentage of capillary water absorption in all samples. Capillary water absorption results were more significant than water absorption results due to the bacterial healing of the samples by spraying. This occurred due to the bacteria being more effective on the surface layer of the samples. The effect of bacteria was highest in the H8 samples because the porosity was greater and caused higher capillary water absorption. A6 bacteria had reasonable recovery rates overall in all samples. Similarly, **Figure 3. 8** shows the capillary absorption coefficient of samples exposed to high temperature.

Table 3. 3 The capillary water absorption for samples exposed to high temperature

Seri	Time v(second)									
	17	24	35	42	60	85	104	120	134	294
H4-SP	0.7	1.4	2.3	3	4.5	6.6	7.9	8.9	9.8	17.8
H4-A6	0.5	0.8	1.2	1.5	2.2	3.1	3.7	4.3	4.8	9.6
H4-C15	0.5	0.9	1.4	1.9	2.9	4.4	5.4	6.2	7.1	15.5
H4-D25	0.5	1.1	1.9	2.6	4.2	6.2	7.4	8.5	9.5	18.1
H4-E31	0.4	0.7	1.2	1.6	2.3	3.4	4.1	4.7	5.4	11.1
H4-E34	0.5	1	1.8	2.4	3.6	5.4	6.5	7.5	8.4	15.4
H6-SP	1.5	2.6	4	4.9	6.8	9.4	11	12.4	13.5	22.7
H6-A6	1.7	2.9	4.4	5.5	7.9	11.1	13.2	15	16.6	28.3
H6-C15	1.6	2.7	4.1	5.2	7.2	10.1	11.9	13.3	14.5	22.9
H6-D25	1.2	2.2	3.6	4.6	6.7	9.6	11.5	13	14.4	24.4
H6-E31	1.2	2.1	3.3	4.3	6.2	9	10.6	12	13.3	22.6
H6-E34	1.3	2.3	3.7	4.7	6.9	9.9	11.9	13.5	15	27.4
H8-SP	2.4	3.9	5.7	7	10	14.3	17.2	19.6	21.6	35.8
H8-A6	2.5	4.3	6.7	8.5	12	16.9	19.9	22.3	24.5	40
H8-C15	2.6	4.5	6.7	8.5	12	17	20.2	22.7	24.7	38.2
H8-D25	2.5	4.3	6.5	8.2	11.9	17.3	20.6	23.2	25.5	39.8
H8-E31	2.7	4.6	7.2	9.1	13.2	18.8	22.2	24.7	26.9	40.9
H8-E34	2.4	4.2	6.4	8.1	11.7	16.5	19.5	21.9	24.1	40.1

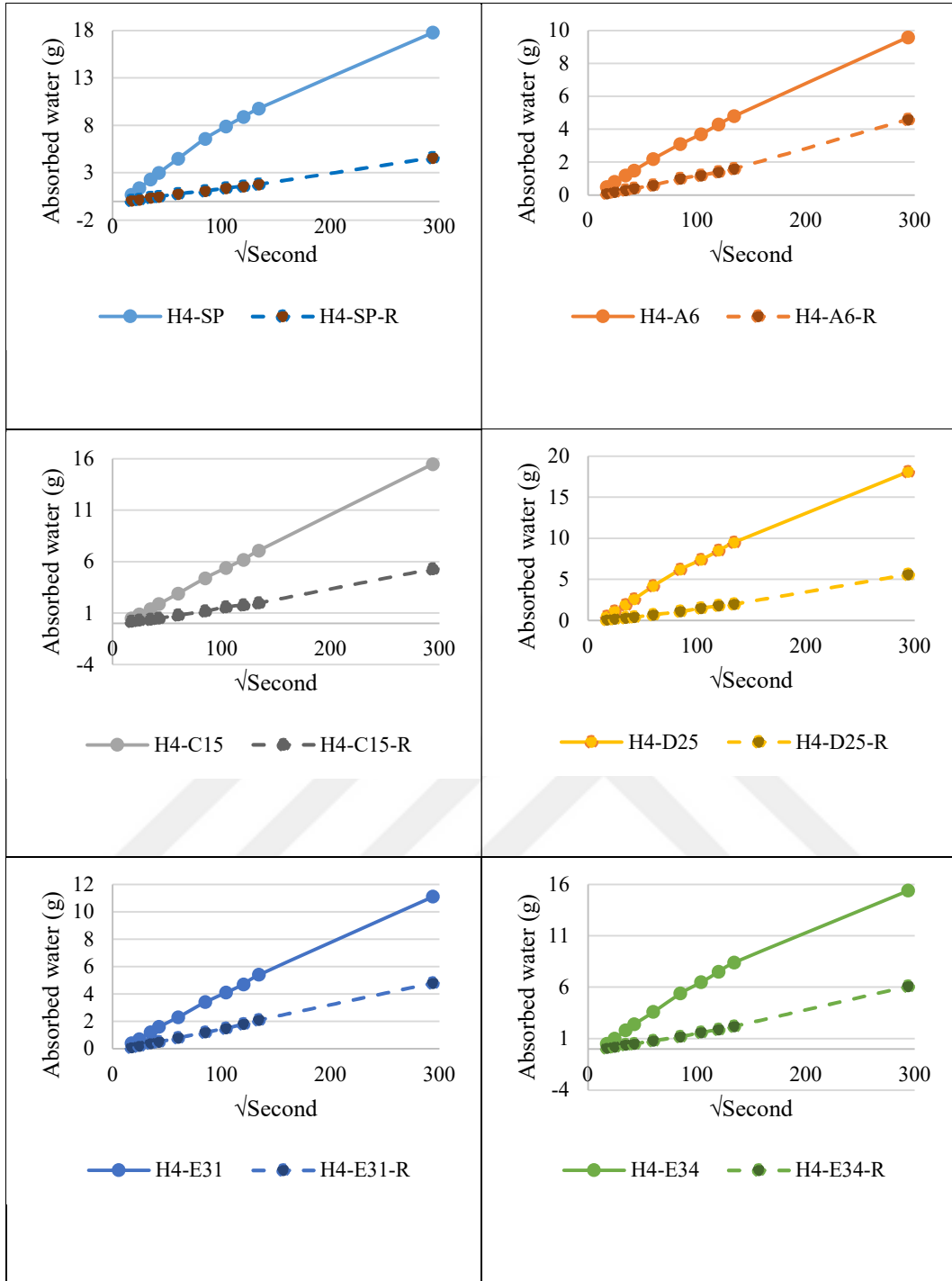


Figure 3. 5 The capillary water absorption for samples exposed to 400 °C

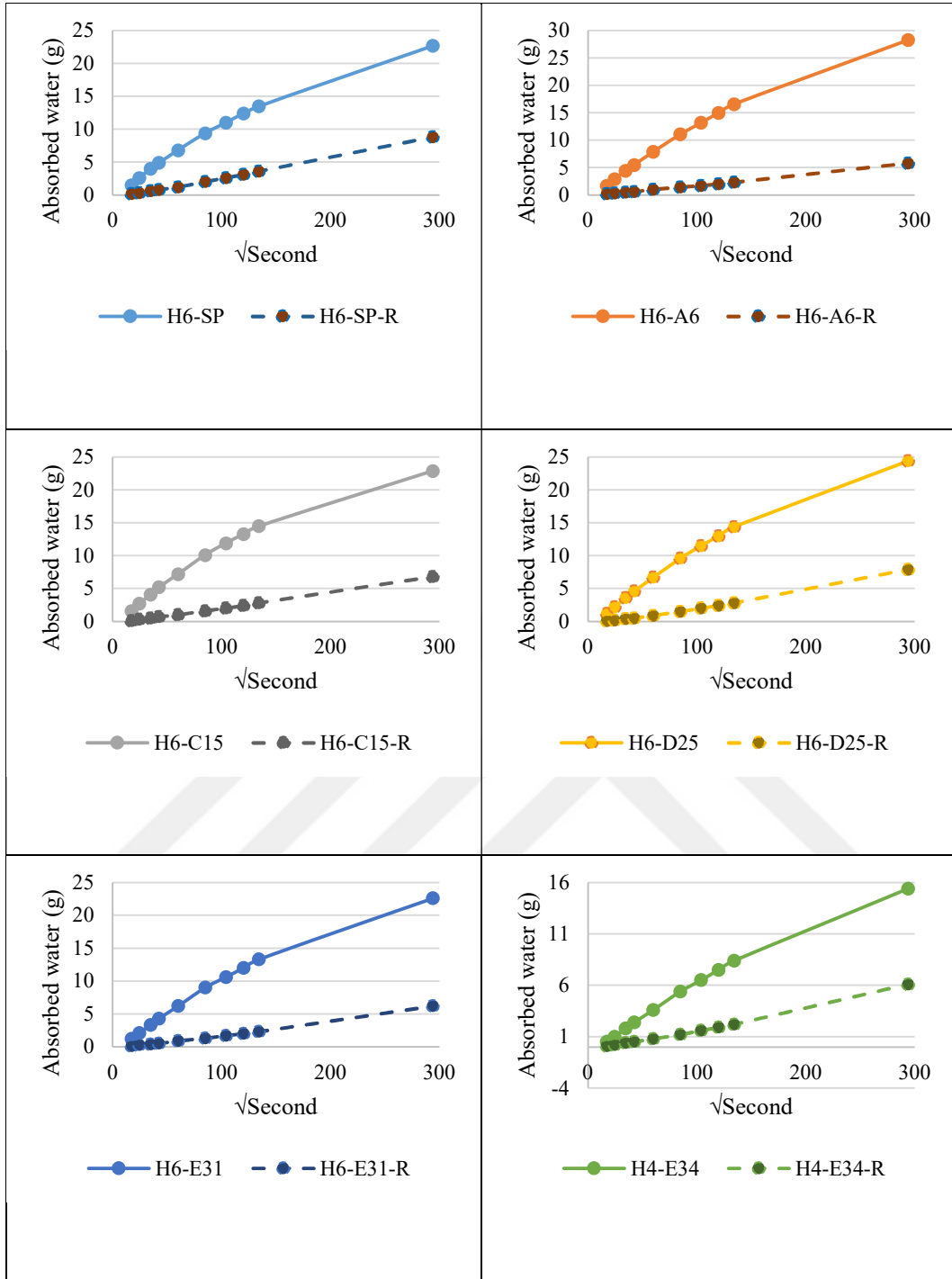


Figure 3. 6 The capillary water absorption for samples exposed to 600 °C

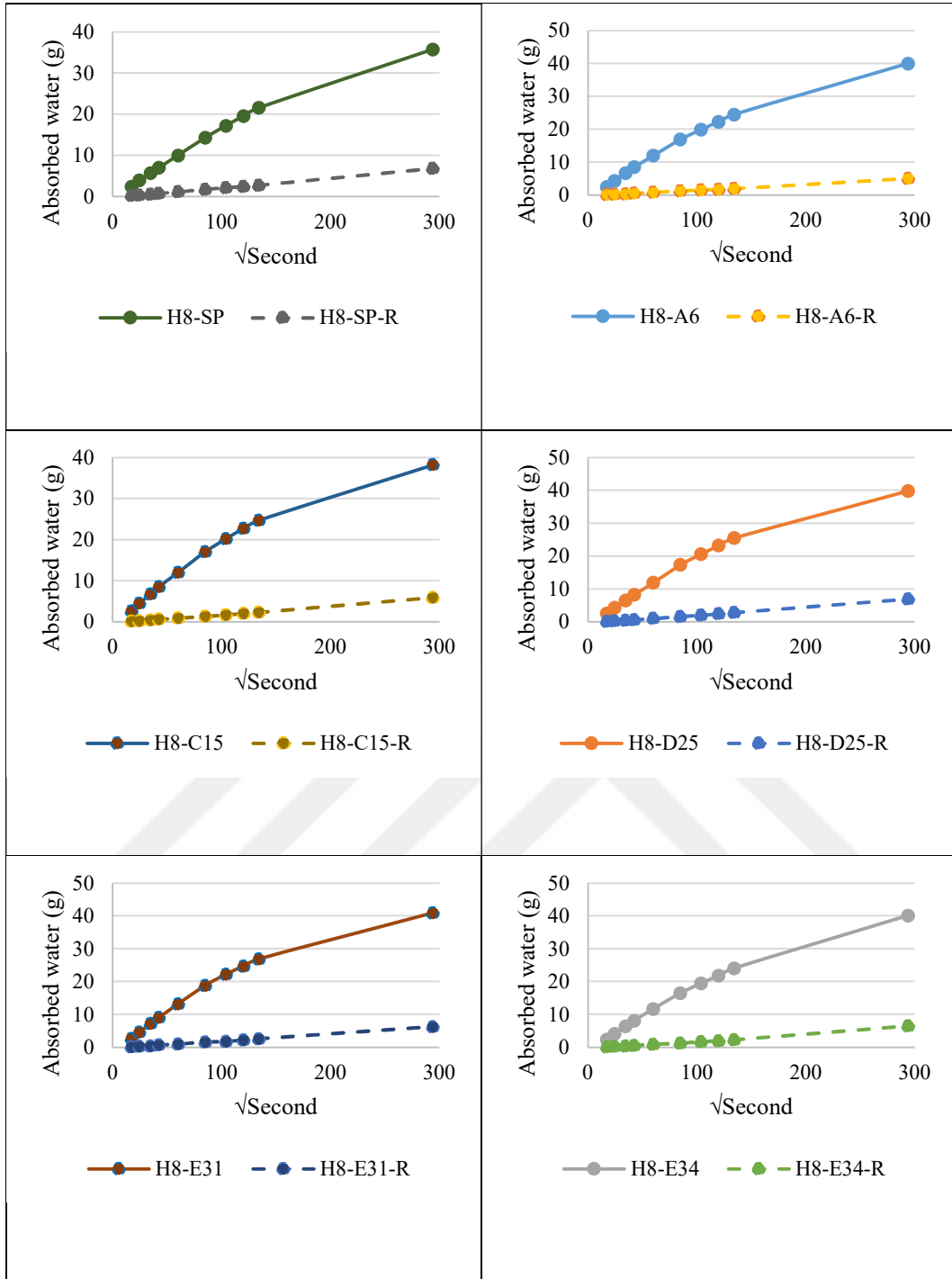


Figure 3. 7 The capillary water absorption for samples exposed to 800 °C

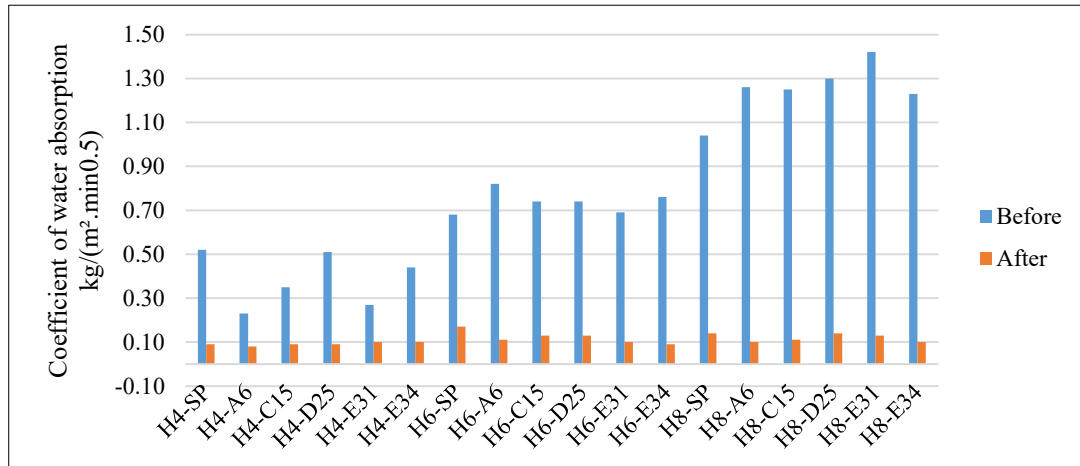


Figure 3. 8 Coefficient of water absorption

For samples exposed to the freeze-thaw effect, capillary water absorption increased due to these cycles [105]. The capillary water absorption increases between 13.2% and 170.8% in the samples healed with E31 and SP bacteria, respectively, as shown in **Figure 3. 9** and **Figure 3. 10**. It can be observed that the capillary water absorption increases due to cracking and deterioration of the surface of the samples. It can be observed that all samples containing bacteria, regardless of their strains, have a lower capillary water absorption rate than the control samples, which confirms the efficiency of the bacteria in the case of addition during mixing [72]. This is because bacteria cause changes in the microstructure, especially in the total porosity, the number of interconnected pores, and the pore sizes due to the formation of calcium carbonate in the pores and capillaries [108].

Table 3. 4 The capillary water absorption for samples exposed to Freeze-Thaw

Seri	Time $\sqrt{(\text{second})}$										
	8	17	24	35	42	60	85	104	120	134	294
FB-SP	0.2	0.4	0.5	0.6	0.7	0.8	1.0	1.1	1.2	1.3	1.4
FB-A6	0.2	0.3	0.4	0.5	0.6	0.7	0.9	1.0	1.1	1.1	1.2
FB-C15	0.3	0.5	0.6	0.7	0.8	0.9	1.0	1.3	1.5	1.8	2.2
FB-D25	0.4	0.5	0.6	0.7	0.7	0.9	1.1	1.2	1.4	1.5	2.0
FB-E31	0.2	0.3	0.4	0.5	0.6	0.7	1.0	1.2	1.3	1.4	1.9
FB-E34	0.3	0.4	0.5	0.6	0.6	0.8	1.0	1.1	1.2	1.3	1.5
FB-SP-R	0.2	0.3	0.4	0.5	0.6	0.8	1.1	1.3	1.4	1.6	2.8
FB-A6-R	0.2	0.3	0.3	0.4	0.5	0.6	1.0	1.2	1.4	1.5	3.3
FB-C15-R	0.3	0.4	0.4	0.7	1.0	0.8	1.1	1.3	1.5	1.6	2.8
FB-D25-R	0.4	0.6	0.6	0.8	0.9	0.6	1.5	1.7	2.0	2.2	3.9
FB-E31-R	0.1	0.2	0.3	0.3	0.4	0.5	0.8	0.8	1.0	1.1	2.1
FB-E34-R	0.3	0.3	0.3	0.3	0.4	0.5	0.8	0.8	0.9	1.1	2.2
F-Control-R	1.0	0.8	1.0	1.2	1.4	2.0	2.8	3.0	3.3	3.6	5.9

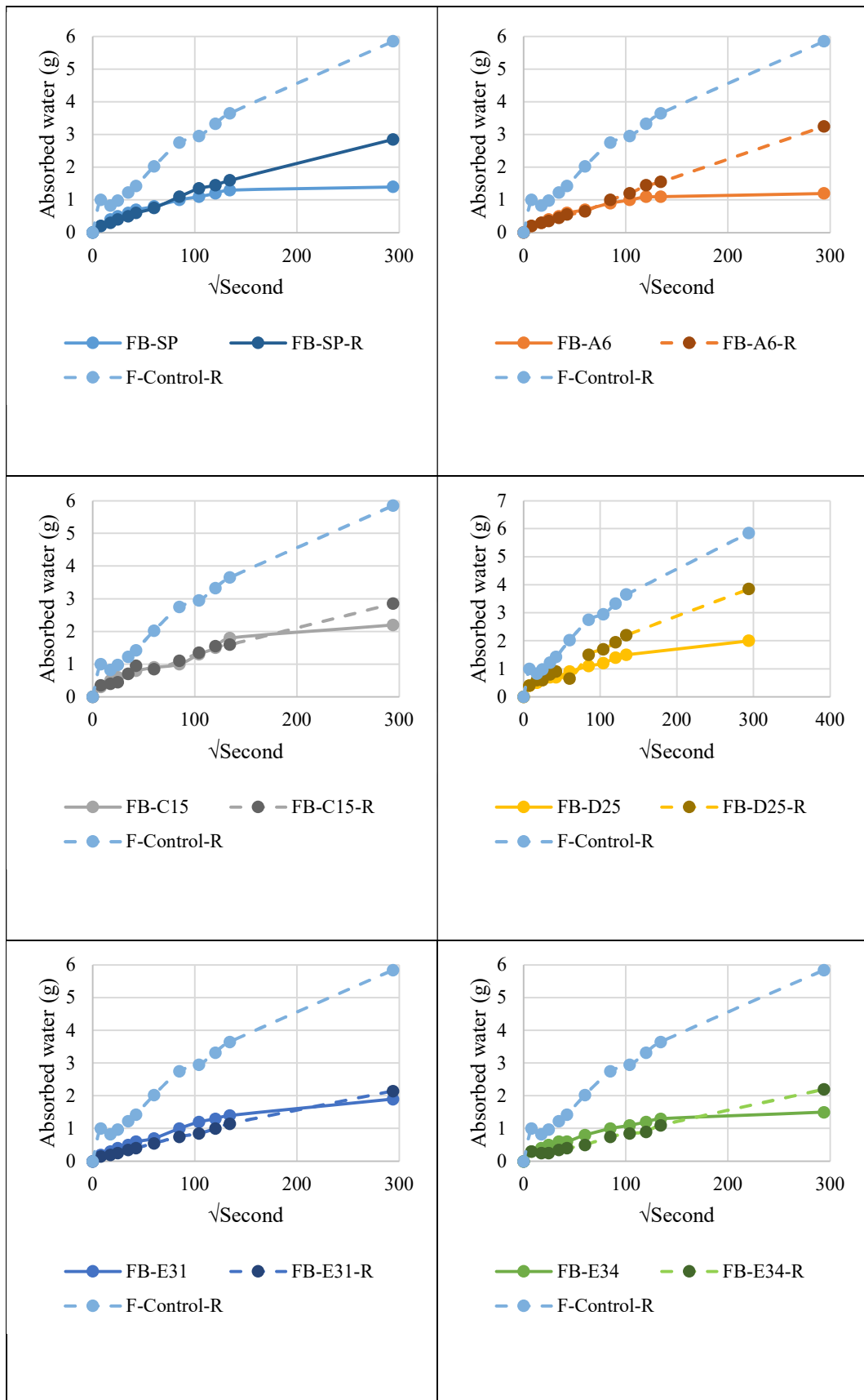


Figure 3. 9 The capillary water absorption for samples exposed to Freeze-Thaw

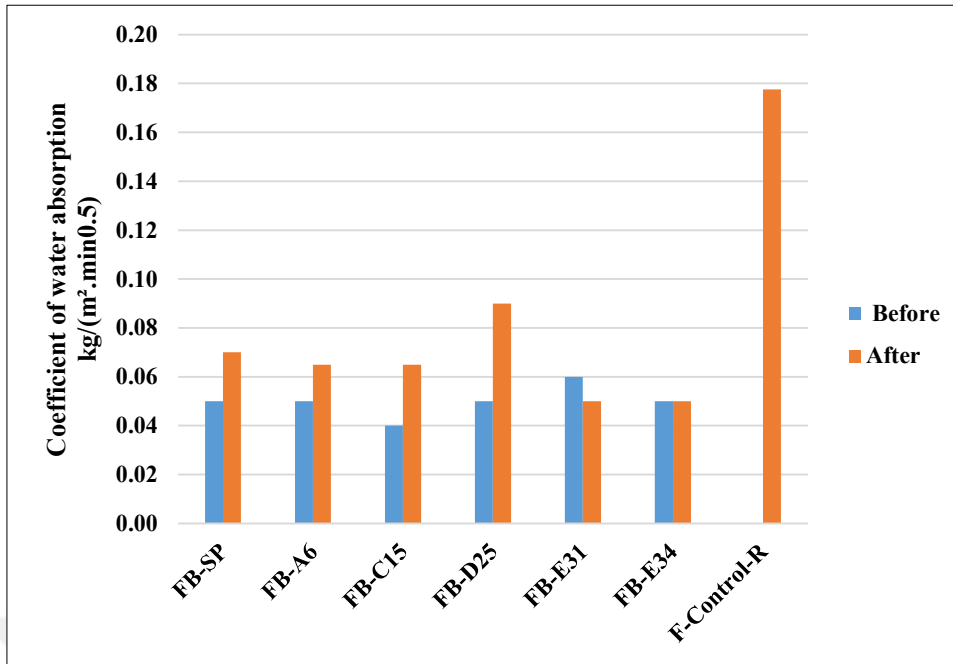


Figure 3. 10 Coefficient of water absorption for samples exposed to Freeze-Thaw

3.4 UPV Test

The percentage of recovery for the samples was considered as the ratio of the UPV values after and before healing. The UPV test can be used to examine the concrete structure by measuring the UPV through the body of the material. Exposing concrete to high temperatures leads to the disintegration of structural bonds and thus to the formation of cracks and expansion, increasing voids in the concrete structure, which reduces the UPV [109], [110]. Therefore, UPV was used to determine the recovery of concrete in bacterial healing experiments of concrete [73]. UPV was also used to determine the bacterial healing efficiency of cement mortar by adding two strains of bacteria in an impregnated form with the fiber and adding the nutrient medium with it or through curing in water. This study showed a higher efficiency for adding bacteria during mixing [111]. The results in the samples exposed to a temperature of 400 °C show that the healing percentage ranged between 95.8 % for TSB and 99% for SP bacteria. For the samples exposed to a temperature of 600 °C, the healing percentage ranged between 97.1% for TSB and 101.3% for SP bacteria. In the samples exposed to a temperature of 800 °C, the healing percentage ranged between 88.1% for E34 bacteria and 95.5% for SP bacteria, as shown in **Figure 3. 11**. The results show generally high recovery rates for all samples at different temperatures and strains of bacteria. Increased UPV indicates the filling of pores and cracks in cement mortar with calcium carbonate

precipitated by bacteria and can, therefore, be considered a healing rate [112]. Since the H6 samples had connected and deeper cracks than H4, the bacterial-healing percentage of H6 samples is greater than that of H4 samples according to UPV values. On the other hand, H8 samples had significant deterioration and large pores. This led to the cracks of H8 samples hardly healing. For samples subjected to freeze-thaw cycles, the recovery rate ranged from 93% for control samples without bacteria to 96% for D25, E31, and E34, as shown in **Figure 3. 12**. The results of samples subjected to freeze-thaw cycles show that the bacteria-containing samples are resistant to degradation compared to the control samples. The D25, E31, and E34 bacteria were also efficient in bacterial healing. This is due to the bacteria's ability to withstand freezing and thawing conditions and the precipitation of calcium carbonate [113].

Table 3. 5 UPV test results for samples exposed to high-temperature

Seri	UPV (m/s)			
	28 days	After high temperture	After healing	Healing %
H4-SP	4389.57	3686.64	4351.77	99%
H4-A6	4401.65	3742.69	4289.54	97%
H4-C15	4401.65	3778.04	4259.09	97%
H4-D25	4389.57	3733.96	4347.83	99%
H4-E31	4389.57	3836.93	4367.61	99%
H4-E34	4389.57	3732.50	4278.07	97%
ATTCC4	4393.59	3749.77	4341.93	99%
TSB4	4393.59	3749.77	4210.53	96%
H6-SP	4332.13	2442.75	4379.56	101%
H6-A6	4341.93	2411.45	4289.54	99%
H6-C15	4324.32	2461.54	4229.07	98%
H6-D25	4355.72	2354.67	4330.18	99%
H6-E31	4389.57	2308.80	4347.83	99%
H6-E34	4351.77	2150.54	4229.07	97%
ATTCC6	4348.61	2361.28	4312.67	99%
TSB6	4348.61	2361.28	4221.64	97%
H8-SP	4407.71	2903.81	4210.53	96%
H8-A6	4389.57	2939.38	4192.14	96%
H8-C15	4371.58	2930.40	4116.64	94%
H8-D25	4389.57	2961.14	4113.11	94%
H8-E31	4386.57	2972.14	4113.11	94%
H8-E34	4377.56	3078.90	3855.42	88%
ATTCC8	4388.37	2963.27	4150.45	95%
TSB8	4388.37	2963.27	4035.31	92%

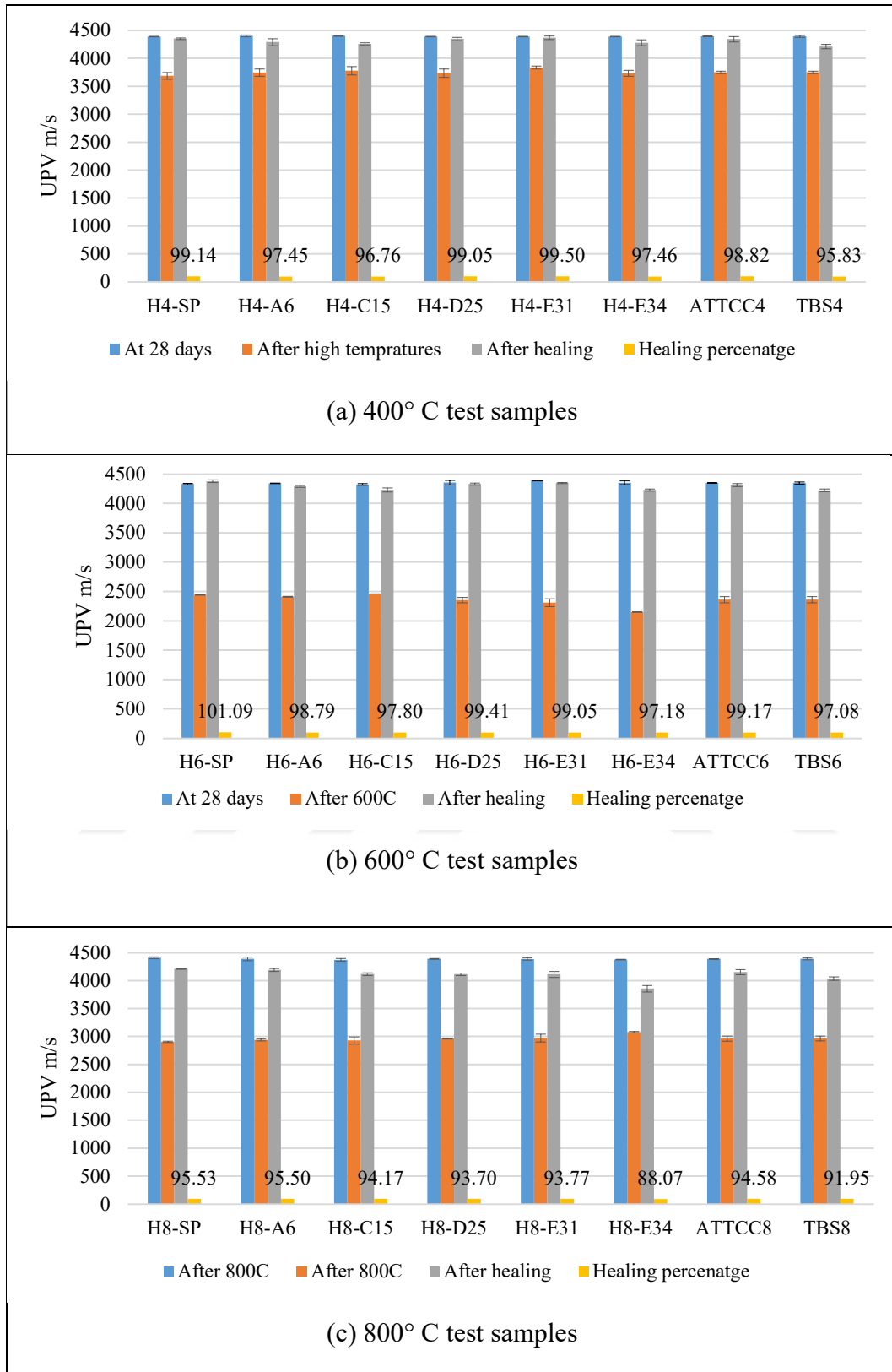
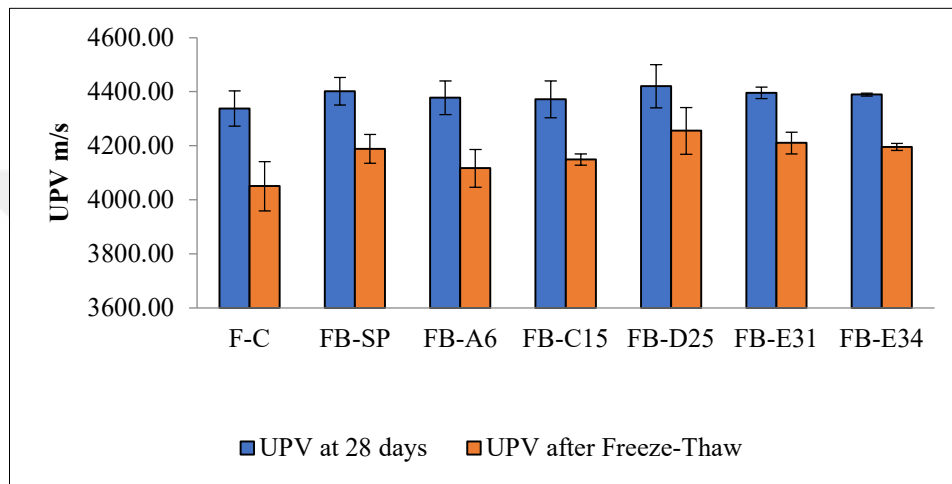


Figure 3. 11 UPV test results for samples exposed to high-temperature

Table 3. 6 UPV test results for samples exposed to Freeze-Thaw cycles

Seri	UPV (m/s)		Healing %
	28 Days	Freeze-Thaw	
F-C	4338.00	4050.63	93%
FB-SP	4401.65	4188.48	95%
FB-A6	4377.56	4116.64	94%
FB-C15	4371.58	4148.66	95%
FB-D25	4419.89	4255.32	96%
FB-E31	4395.60	4210.53	96%
FB-E34	4389.57	4195.80	96%

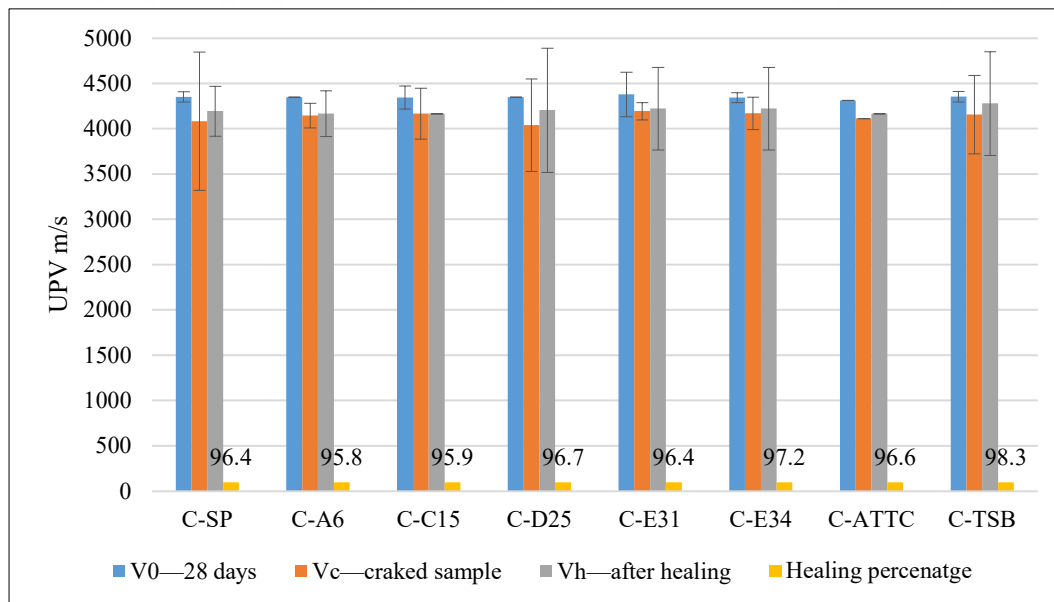
**Figure 3. 12** UPV test results for samples exposed to Freeze-Thaw

Studies on bacterial healing have been conducted using UPV testing of cracks induced by flexural loads [111], [114]. Direct injection healing of cracks induced (150-250 μm) in bacteria-free samples and samples to which bacteria were added during mixing was performed. Free bacteria samples C had recovery rates between 95.8% for A6 bacteria and 97.2% for E34 bacteria. The CB samples with bacteria had recovery rates between 95.7% for C15 bacteria and 98.8% for nutrients with E34 bacteria samples, as shown in **Figure 3. 13** and **Figure 3. 14**. It can be noted that the percentage of recovery is close between CB and C samples. Still, CB samples had better results before cracking, indicating the effect of bacteria in the mixture, reflected in the results after recovery, as they were better than C samples. This is because the calcium carbonate precipitated by bacteria in the pores is less affected by thermal changes than the water, which causes significant expansions [112].

Table 3. 7 UPV test results for cracked C and CB samples

Sample Seri	Before Healing	After Healing	Healing %
C-SP	4352	4194	96.4%
C-A6	4348	4167	95.8%
C-C15	4345	4167	95.9%
C-D25	4348	4204	96.7%
C-E31	4380	4222	96.4%
C-E34	4344	4222	97.2%
C-ATTC	4313	4167	96.6%
C-TSB	4354	4279	98.3%
CB-SP	4392	4250	96.8%
CB-A6	4384	4241	96.8%
CB-C15	4384	4194	95.7%
CB-D25	4408	4336	98.4%
CB-E31	4438	4307	97.0%
CB-E34	4396	4278	97.3%
CB-ATTCB-(CB-SP)	4408	4278	97.1%
CB-TSB- (CB-D25)	4396	4336	98.6%
CB-TSB- (CB-E34)	4390	4336	98.8%
CB-TSB- (CB-C15)	4390	4307	98.1%
CB-TSB- (CB-E31)	4396	4307	98.0%

Figure 3. 13 UPV test results for cracked C samples



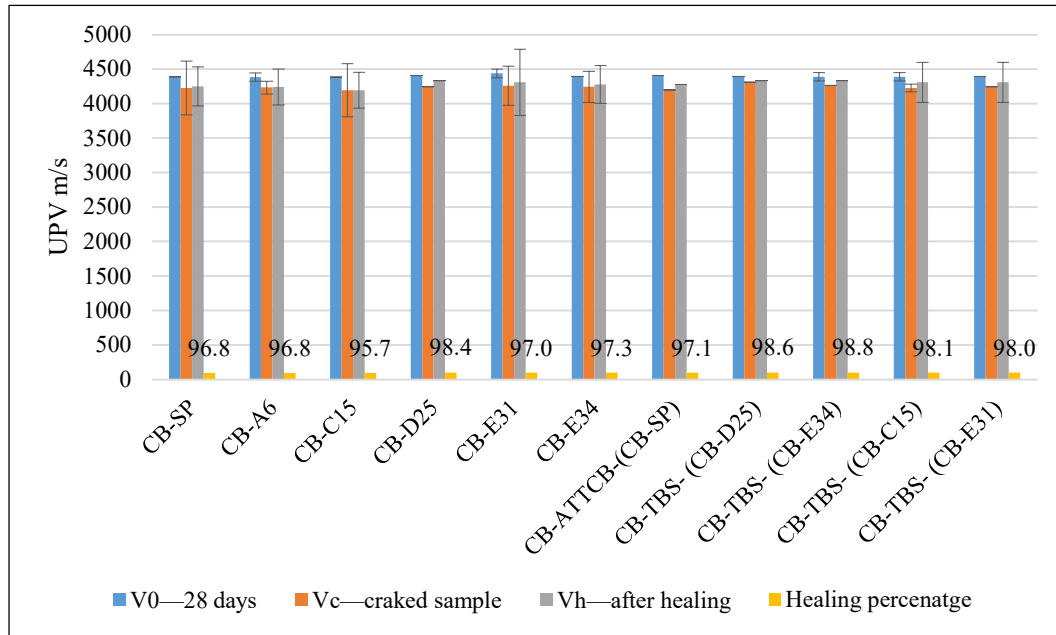


Figure 3. 14 UPV test results for cracked CB samples

3.5 Flexural and Compressive Strength

The compressive strength and flexural strength tests are among the most essential tests in evaluating the quality and properties of concrete. They are among the most important properties that are desired in concrete. Therefore, they are used in research to test concrete and obtain results. Among these researches comes the study of bacterial healing and its effect on restoring these two properties. Nugroho and Satyarno studied the healing of flexural strength in cracks caused by loading and healing by immersion in a bacterial medium, as well as the healing of compressive strength for samples containing fly ash saturated with bacteria [115]. They obtained recovery rates for compressive strength (6-18%) and recovery of 34% for flexural strength. A previous study examined compressive strength and flexural strength to compare three different healing methods (injection, spraying, and immersion) with bacteria, and they found that the injection method is more effective with recovery rates of about 30% for flexural strength and about 10% for compressive strength [73]. The results showed that the percentage of recovery in CB samples that contained bacteria during mixing was more significant than in samples free of bacteria. Recovery ranged from 16% for D25 bacteria to 21% for E31 bacteria in C samples, 19% for E31 bacteria, and 26% for D25 bacteria in CB samples, as shown in **Figure 3. 15**. It was observed that the healing products

enhanced the healed samples' compactness, resulting in the recovery of flexural strength[116], [117].

Table 3. 8 Flexural strength before and after healing in C and CB samples

Seri	Ft Mpa		Heling percentatge
	28 days	After healing	
CSP	7.88	1.56	20%
CA6	7.88	1.35	17%
CC15	7.88	1.92	24%
CD25	7.88	1.26	16%
CE31	7.88	1.67	21%
CE34	7.88	1.59	20%
ATTCC	7.88	1.26	16%
TSB	7.88	1.59	20%
CBSP	7.17	1.51	21%
CBA6	7.17	1.57	22%
CBC15	7.17	1.76	25%
CBD25	7.17	1.84	26%
CBE31	7.17	1.39	19%
CBE34	7.17	1.49	21%
ATTCC	7.17	1.67	23%
TSB-D25	7.17	1.52	21%
TSB-E34	7.17	1.64	23%
TSB-E31	7.17	1.66	23%
TSB-A6	7.17	1.54	21%
TSB-C15	7.17	1.48	21%

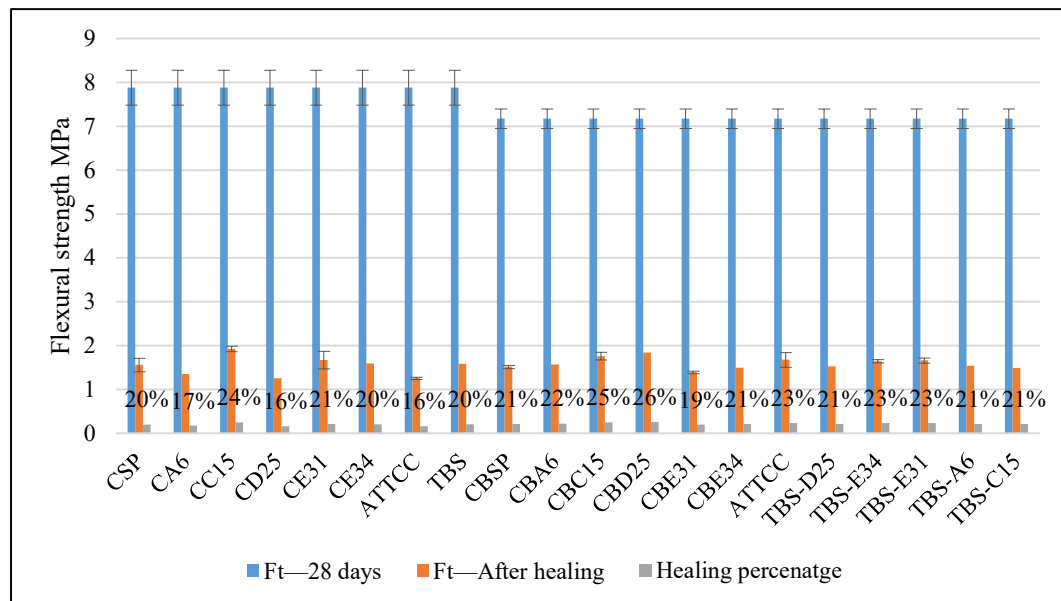


Figure 3. 15 Flexural strength before and after healing in C and CB samples

For the samples subjected to high temperature, the flexural strength results indicate a decrease of 2% in the H4 samples, 48% in the H6 samples, and 73% in the H8 samples, as shown in **Figure 3. 16**. Similarly, compressive strength decreased by 17% in the H4 samples, 46% in the H6 samples, and 64% in the H8 samples, as shown in **Figure 3. 17**. The decrease in the compressive strength results from the drying and disintegration of the concrete and from the expansion that breaks the bonds and increases the pores [109], [118]. Using samples subjected to high temperatures is uncommon for studying bacterial healing. Still, the heat cracks in samples of different sizes can be used to research and compare the efficiency of various bacteria in healing. The flexural strength recovery ranged between 105% for D25 and 112% for E34 in H4, 70% for C15 and 86% for D25 in H6, and 58% for SP and 68% for E31 in H8 samples, as shown in **Figure 3. 16**.

Table 3. 9 Flexural strength before and after healing in H4, H6, and H8 samples

Seri	Flexural Strength Ft MPa			Healing percentage
	28 Days	After Heating	After Healing	
H4-SP	8.59	8.31	9.59	112%
H4-A6	8.59	8.31	9.02	105%
H4-C15	8.59	8.31	9.69	113%
H4-D25	8.59	8.31	9.00	105%
H4-E31	8.59	8.31	9.32	109%
H4-E34	8.59	8.31	9.78	114%
ATTCC4	8.59	8.31	9.68	113%
TSB4	8.59	8.31	9.00	105%
H6-SP	8.61	4.62	6.29	73%
H6-A6	8.61	4.62	7.10	82%
H6-C15	8.61	4.62	6.07	70%
H6-D25	8.61	4.62	7.44	86%
H6-E31	8.61	4.62	6.79	79%
H6-E34	8.61	4.62	6.52	76%
ATTCC6	8.61	4.62	5.19	60%
TSB6	8.61	4.62	5.71	66%
H8-SP	8.14	2.45	4.76	58%
H8-A6	8.14	2.45	5.15	63%
H8-C15	8.14	2.45	5.16	63%
H8-D25	8.14	2.45	5.55	68%
H8-E31	8.14	2.45	5.51	68%
H8-E34	8.14	2.45	5.41	67%
ATTCC8	8.14	2.45	5.05	62%
TSB8	8.14	2.45	4.85	60%

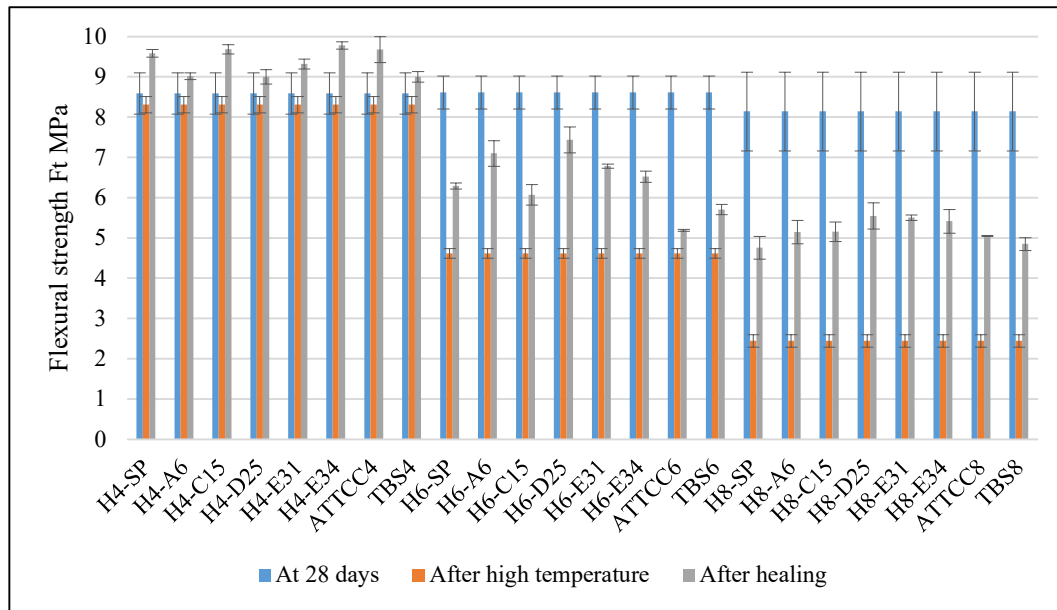


Figure 3. 16 Flexural strength before and after healing in H4, H6, and H8 samples. The compressive strength results of the samples showed that the recovery ranged between 100% for C15 and 103% for A6 in H4 samples, 79% for C15 and 86% for SP in H6 samples, and 80% for E31 and 85% for C15 in H8 samples as shown in **Figure 3. 17**. The flexural strength and compressive strength of H4 samples continued to improve as the deterioration was small, and the effect of bacteria here was not considered essential. As for H6 and H8 samples, bacterial healing played a clear role in healing the flexural strength and rehydrating the cementitious materials disintegrated due to high temperature. This indicates that the cement mortar has healed and not sealed.

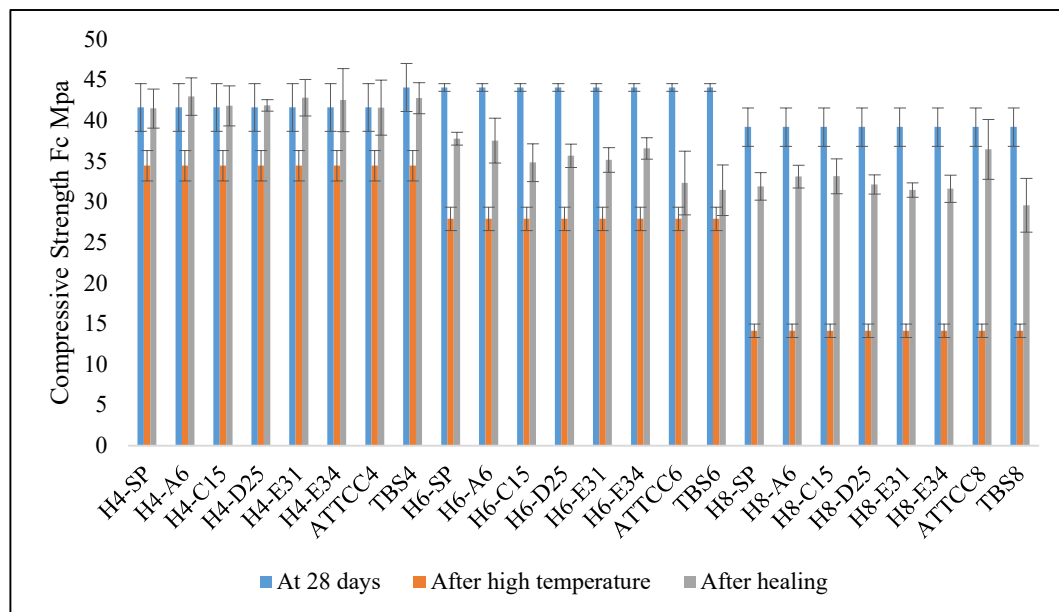


Figure 3. 17 Compressive strength before and after healing in H4, H6, and H8 samples

Table 3. 10 Compressive strength before and after healing in H4, H6, and H8 samples

Seri	Flexural Strength Ft MPa			Healing precentage
	28 Days	After Heating	After Healing	
H4-SP	41.63	34.45	41.49	100%
H4-A6	41.63	34.45	42.97	103%
H4-C15	41.63	34.45	41.83	100%
H4-D25	41.63	34.45	41.87	101%
H4-E31	41.63	34.45	42.82	103%
H4-E34	41.63	34.45	42.53	102%
ATTCC4	41.63	34.45	41.61	100%
TSB4	44.08	34.45	42.76	97%
H6-SP	44.08	27.93	37.78	86%
H6-A6	44.08	27.93	37.55	85%
H6-C15	44.08	27.93	34.84	79%
H6-D25	44.08	27.93	35.66	81%
H6-E31	44.08	27.93	35.17	80%
H6-E34	44.08	27.93	36.57	83%
ATTCC6	44.08	27.93	32.33	73%
TSB6	44.08	27.93	31.46	71%
H8-SP	39.22	14.16	31.91	81%
H8-A6	39.22	14.16	33.12	84%
H8-C15	39.22	14.16	33.16	85%
H8-D25	39.22	14.16	32.14	82%
H8-E31	39.22	14.16	31.46	80%
H8-E34	39.22	14.16	31.63	81%
ATTCC8	39.22	14.16	36.46	93%
TSB8	39.22	14.16	29.59	75%

It is known from previous research that freeze-thaw cycles can lead to significant deterioration in the mechanical properties of concrete, especially its compressive and flexural strength [119]. In this research, the test was applied to samples at 28 days of age to observe the development of flexural and compressive strengths. Although the bacteria-free samples (F-C) had a higher flexural strength before healing, the same samples had almost the lowest flexural strength after healing. Similarly, the compressive improved compared to the control samples. This confirms the role of bacteria both in the initial age of the samples and during the freeze-thaw cycles. This is due to the ability of bacteria to precipitate CaCO_3 starting from mixing and the bacteria continuing their role in precipitating CaCO_3 during freezing and thawing [113]. For samples subjected to freeze-thaw cycles,

flexural strength ranged from 31% for E31 bacteria to 62% for D25 bacteria, as shown in **Figure 3. 18**. Compressive strength ranged from 14% for E34 bacteria to 36% for SP bacteria, as shown in **Figure 3. 19**.

Table 3. 11 Flexural strength and Compressive strength before and after healing in samples F, FB

Seri	Ft MPa		Ft Healing %	Fc Mpa		Fc Healing %
	28 Days	After 300 cycles		28 Days	After 300 cycles	
F-C	7.99	10.52	32%	39.92	48.71	22%
FB-SP	7.17	11.57	61%	41.43	56.49	36%
FB-A6	7.17	10.85	51%	47.29	57.24	21%
FB-C15	7.34	12.45	70%	43.32	56.18	30%
FB-D25	7.34	11.92	62%	44.68	58.36	31%
FB-E31	7.16	9.41	31%	43.56	53.19	22%
FB-E34	7.18	11.36	58%	43.72	50.00	14%

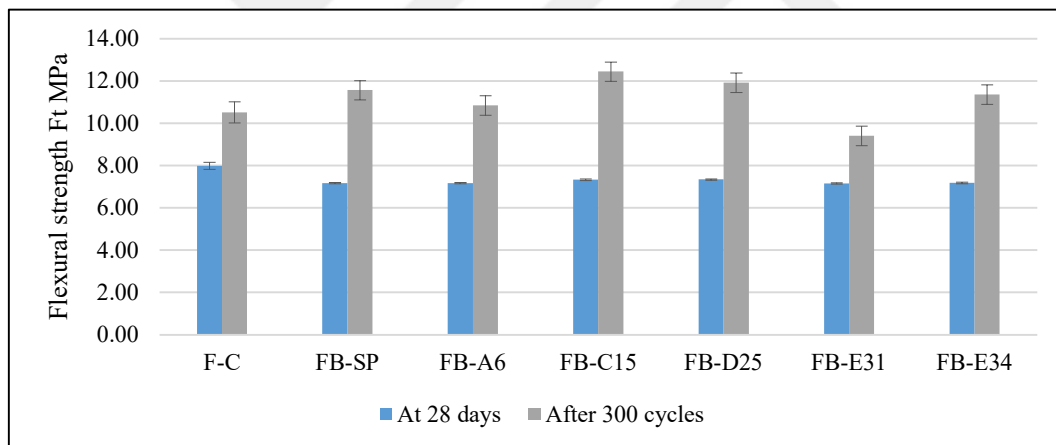


Figure 3. 18 Flexural strength before and after healing in samples F, FB

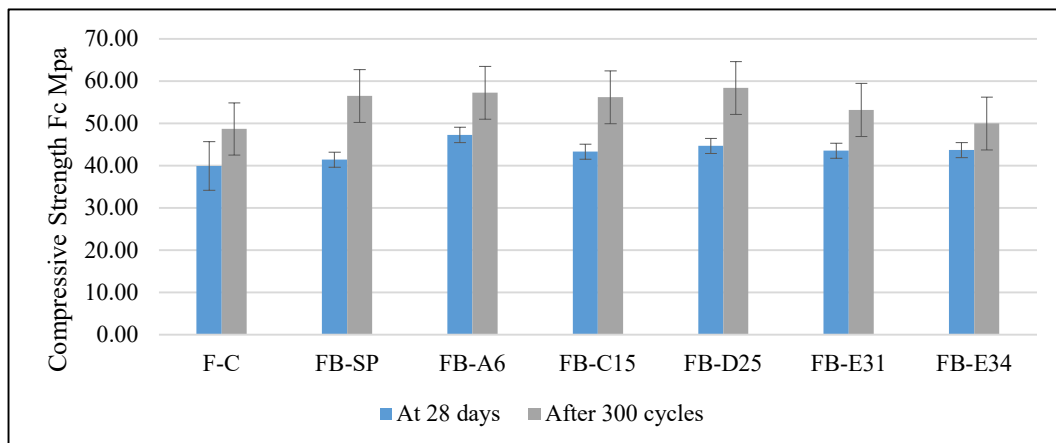


Figure 3. 19 Compressive strength before and after healing in samples F, FB

3.6 Statistical analysis of results

The test results for all sample series were analyzed by arranging the samples in each test in descending order, with the most effective sample receiving a score of 8 and the least effective receiving a score of 1. There are six strains of bacteria and two strains of bacterial nutrient medium. All samples in all tests were given a number according to the result of each test. The arithmetic mean of the sum of the numbers obtained by each sample was calculated, and the sample that received the highest mean was considered the most efficient in terms of the strain of bacteria. The standard deviation was also calculated to determine the homogeneity of the results for the samples. The statistical analysis in **Table 3. 12** shows that the bacteria E31 had rank (1) with an arithmetic mean of 4.96, a total score of 119, and a standard deviation of 1.73.

Similarly, A6 bacteria had rank (2) with an arithmetic mean of 4.67, a total score of 112, and a standard deviation of 2.62. At the same time, SP bacteria had rank (3) with an arithmetic mean of 4.58, a total score of 110, and a standard deviation of 2.16. This indicates that E31 and A6 bacteria are more efficient than SP bacteria in cement mortar healing, and therefore, they can be considered as alternatives that can be used.

Table 3. 12 Statistical analysis of test results

Seri		Bacteria samples and ranking							
	Test	SP	A6	C15	D25	E31	E34	Sterile medium	
								ATCC	TSB
H-400	WA	6	8	7	1	5	4	2	3
	CWA	8	4	7	6	3	5		
	UPV	6	3	2	7	8	4	5	1
	FC	2	8	4	5	7	6	3	1
	Ft	5	3	7	2	4	8	6	1
H-600	WA	1	7	3	4	6	5	8	2
	CWA	3	8	5	4	6	7		
	UPV	8	4	3	7	5	2	6	1
	FC	8	7	3	5	4	6	2	1
	Ft	4	7	3	8	6	5	1	2
H-800	WA	3	8	7	2	6	1	5	4
	CWA	3	8	6	4	7	5		
	UPV	8	7	5	3	4	1	6	2
	FC	4	6	7	5	2	3	8	1
	Ft	1	4	5	8	7	6	3	2

Table 3. 13 Statistical analysis of test results (Continued)

Seri	Test	Bacteria samples and ranking							
		SP	A6	C15	D25	E31	E34	Sterile medium	
								ATCC	TSB
C	UPV	3	1	2	6	4	7	5	8
	Ft	4	3	8	2	7	6	1	5
CB	UPV	3	2	1	7	4	6	5	8
	Ft	3	4	7	8	1	2	6	5
FB	WA	6	5	2	1	4	3		
	CWA	3	1	4	2	6	5		
	UPV	3	1	2	6	5	4		
	FC	6	2	4	5	3	1		
	Ft	4	1	6	2	5	3		
Sum		105	112	110	110	119	105	72	47
Average		4.38	4.67	4.58	4.58	4.96	4.38	4.50	2.94
S. deviation		2.16	2.62	2.10	2.30	1.73	2.02	2.25	2.41
Ranking*		6	2	3	4	1	7	5	8

* 1- The best, 8- The worst

3.7 Microstructural analysis of samples

3.7.1 SEM and EDS

SEM, EDS, and XRD analyses were performed for the H4, H6, H8, F, and FB series. SEM micrographs are a means of examining the microstructural structure of materials in their different states, as is the case in many studies [114], [115], [119]. Since the E34 bacteria-contained sample had the lowest performance ranking among the bacteria strains used in this study, it was selected to be investigated and compared with the SP bacteria-contained sample by SEM and EDS. **Figure 3. 20** shows a 20,000 X magnification SEM image of control and healed samples. **Figure 3. 20 (a)** shows a control sample subjected to a temperature of 400 °C, **Figure 3. 20 (b)** a control sample subjected to a temperature of 600 °C. Cementitious material elements, disintegration of these elements, and thermal cracks can be observed due to exposure to high temperatures [120]. **Figure 3. 20 (c)** and **(d)** show samples subjected to a temperature of 400 °C and healed with SP bacteria. Thermal pores and cracks appear in the matrix of the material, and calcite precipitation can be observed in the form of clear clumps, in addition to the endospores of the SP bacteria, which have sprouted as a result of remaining for a period of time without nutrients before performing the SEM imaging [121]. **Figure 3. 20 (e)** and **(f)** show samples subjected to a temperature of 600°C and healed with E34 bacteria. **Figure 3. 20 (c)** and **(d)** show the calcite precipitates on the matrix of the cementitious mortar samples. Also, **Figure 3. 20 (d)** illustrates the SP endospores and the calcite precipitates by it. Similar studies [9] and [122] show that SP was precipitated calcite, which plays a vital role in filling the pores in the samples. As observed from **Figure 3. 20 (b)**, the control sample exposed to 600 °C has more pores than the control sample exposed to 400 °C, as shown in **Figure 3. 20 (a)**. This can be attributed to the increase in dehydration after 600°C [106], [111]. Thus, it can be observed that the SEM analysis was consistent with the mechanical analysis results. In addition, by investigating the E34-contained samples shown in **Figure 3. 20 (e)** and **(f)**, it was observed that the samples contained E34, the sample with the lower performance ranking, had calcite content. Also, by comparing E34 and SP-contained samples, the calcite content of SP-contained samples was higher than that found in E34-contained samples, as shown in **Figure 3. 20 (c)** and **(d)**. Similarly, Al-Mahdi et al. [123] conducted a study on several strains of bacteria and found

through SEM micrographs that samples containing bacteria exhibited calcium carbonate precipitation compared to control samples. They also observed differences in calcium carbonate precipitation rates among different bacterial species.

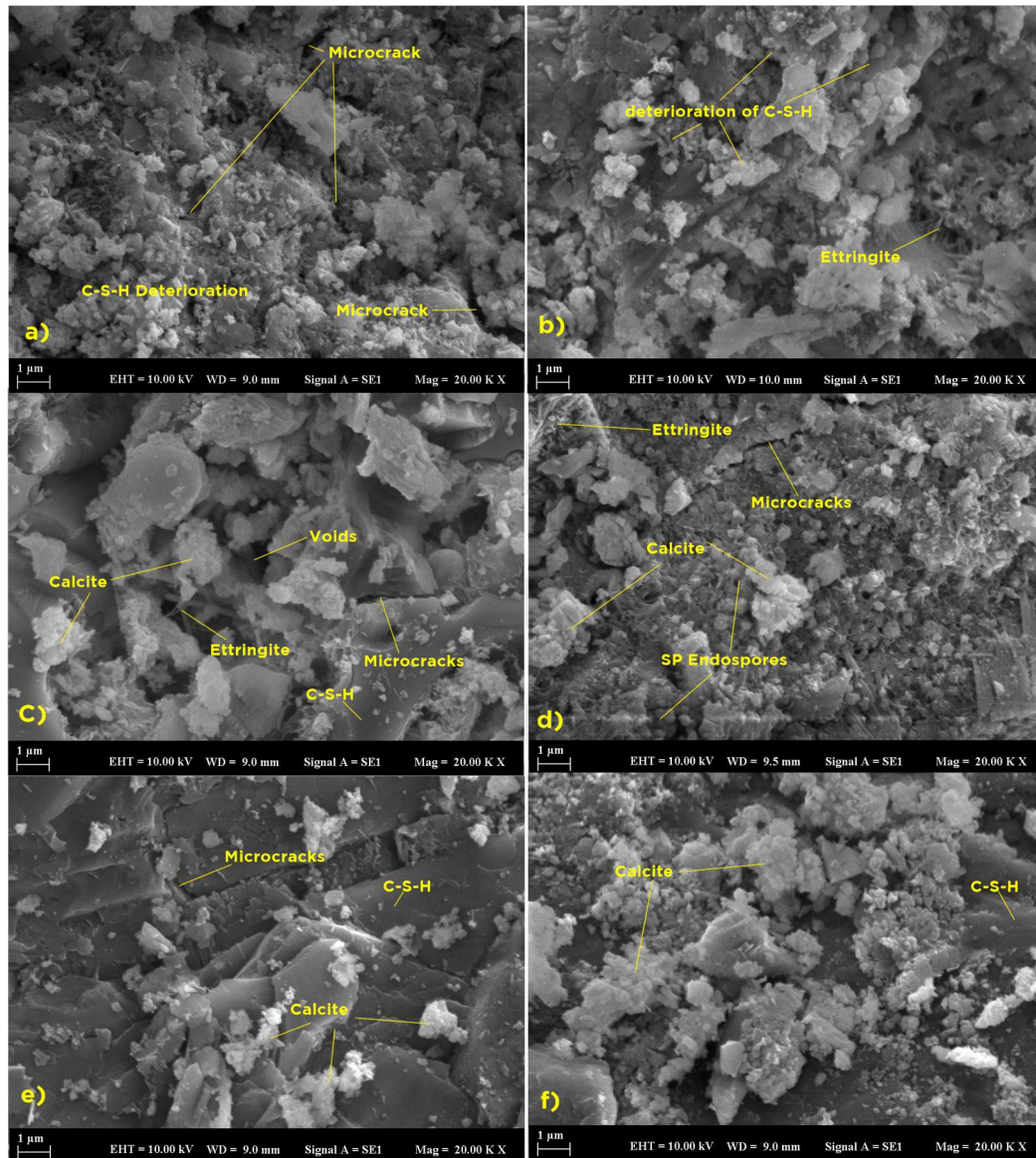


Figure 3. 20 SEM micrographs (20 KX) for samples (a, b) control samples at 400 °C, 600 °C, (c, d) H4 samples with SP bacteria after healing, (e, f) H6 samples with E34 bacteria after healing.

Simultaneously with the SEM analysis, the EDS of the control and the samples healed with SP and E34 bacteria were examined. **Figure 3. 21 (a)** and **(b)** show the EDS spectra of the control samples subjected to 400 °C and 600 °C at four different points in the body of each sample. **Figure 3. 22 (a)** shows the EDS spectra of the SP samples subjected to 400°C, and **Figure 3. 22 (b)** shows the EDS spectra of the

E34 samples subjected to 600°C. Comparing the concentration of calcium, which is the main element of calcite in the control samples, with the samples healed with E34 and SP bacteria, a significant increase in calcium concentration can be observed. Similarly, previous research that studied bacterial healing using bacteria confirmed their effect on calcium carbonate precipitation [72], [73], [106]. Where calcium in the control samples reached its peak at (2.6 K-2.79 K) shown in **Figure 3. 21 (a) and (b)**, calcium increased in the samples healed with both E34 and SP bacteria to (4.2 K-7.4 K) shown in **Figure 3. 22 (a) and (b)**, which is equivalent to an increase of 160%. Thus, the calcite content enhanced the compactness of the structure of the healed samples by filling the pores. This enhancement shown in the EDS analysis was in the same line as the enhancement found in the results of the mechanical tests. Seifan et al. indicate that the initial compositions of the bio-precipitates are similar to pure CaCO_3 crystals, confirming that the resulting particles were produced by bacteria [124].

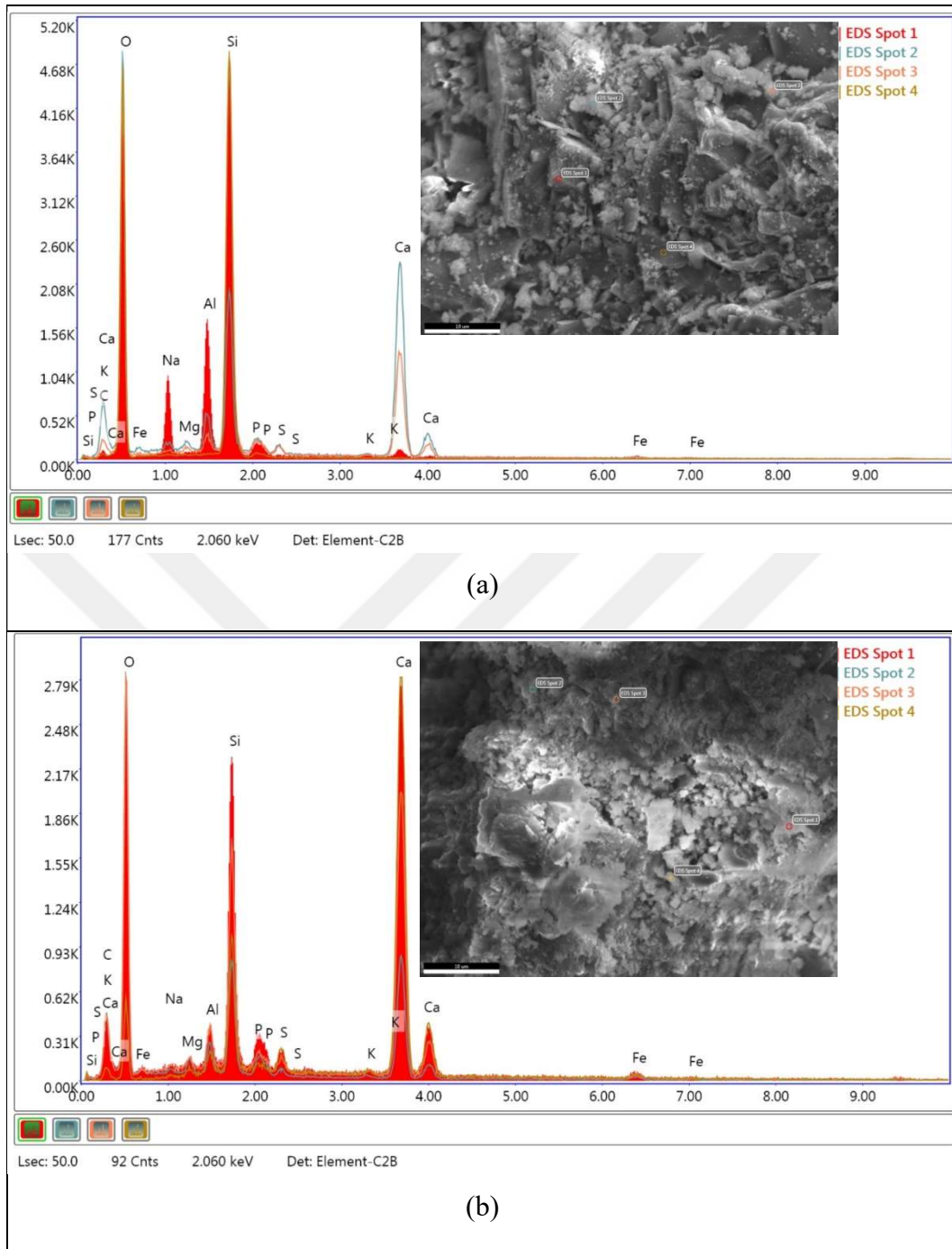


Figure 3. 21 EDS spectroscopy of the (a, b) control samples at 400 °C, 600 °C before healing

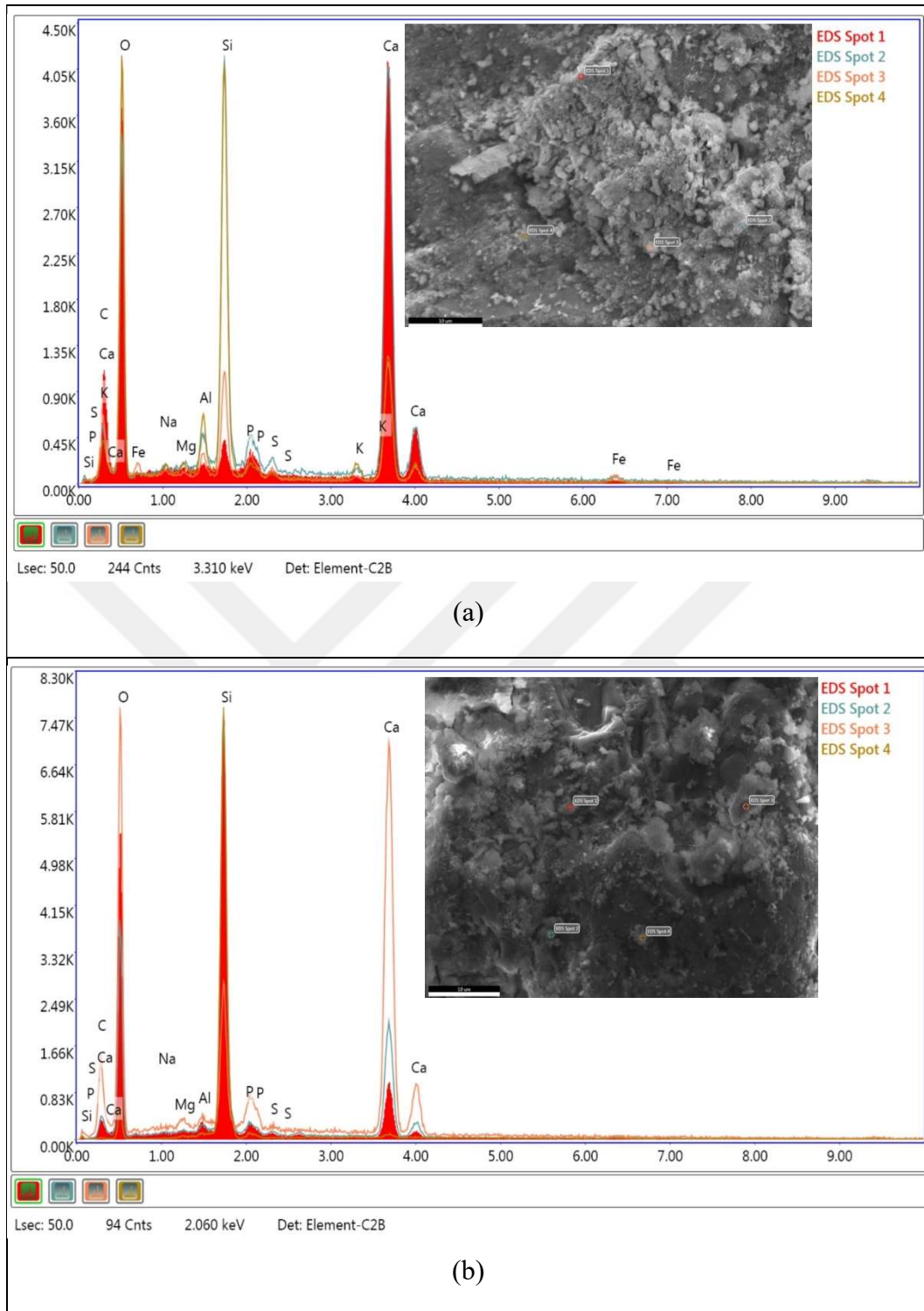


Figure 3. 22 EDS spectroscopy of (a) H4 samples with SP bacteria after healing, and (b) H6 samples with E34 bacteria after healing.

3.7.2 XRD analysis

XRD analysis is distinguished from EDS by its ability to determine the crystal structure of materials and, thus, the different compositions and their proportions in the matrix. Many studies have used this technique to assess the development of the crystal structure of cementitious matrix in bacterial healing experiments to confirm the formation and deposition of calcium carbonate [106], [125], [126]. XRD microscopic analysis was performed for the samples in each experiment, and from each series (H4, H6, H8, F, FB), three samples were taken: the sample in which the bacteria showed the highest efficiency, the sample containing SP bacteria and the control sample in each series. **Figure 3. 23** shows the XRD spectra of the samples for all samples taken from each series. These spectra for H4 samples indicate an increase in peaks at positions ($^{\circ}2\theta$) 23.09, 29.4, and 56 in the samples healed with bacteria E34 and a lesser increase in the SP samples compared to the control samples (H6-M). According to the American Mineralogist Crystal Structure Database Record, these positions indicate calcite, which increases due to bacterial precipitation. For H6 and H8 samples, increasing temperatures lead to the dissociation of C2S and C3S and, consequently, the subsidence of the peaks representing quartz and calcite [103]. For the samples of groups F and FB that were subjected to freeze-thaw cycles, the XRD spectra indicate an apparent increase in calcite compared to the control samples at positions ($^{\circ}2\theta$) 23.09, 29.4, 36, and 56. This increase appears more significant in the SP bacteria samples than in the A6 samples. These results were similar to those obtained in previous studies on the effect of bacteria on samples exposed to freezing and thawing [127].

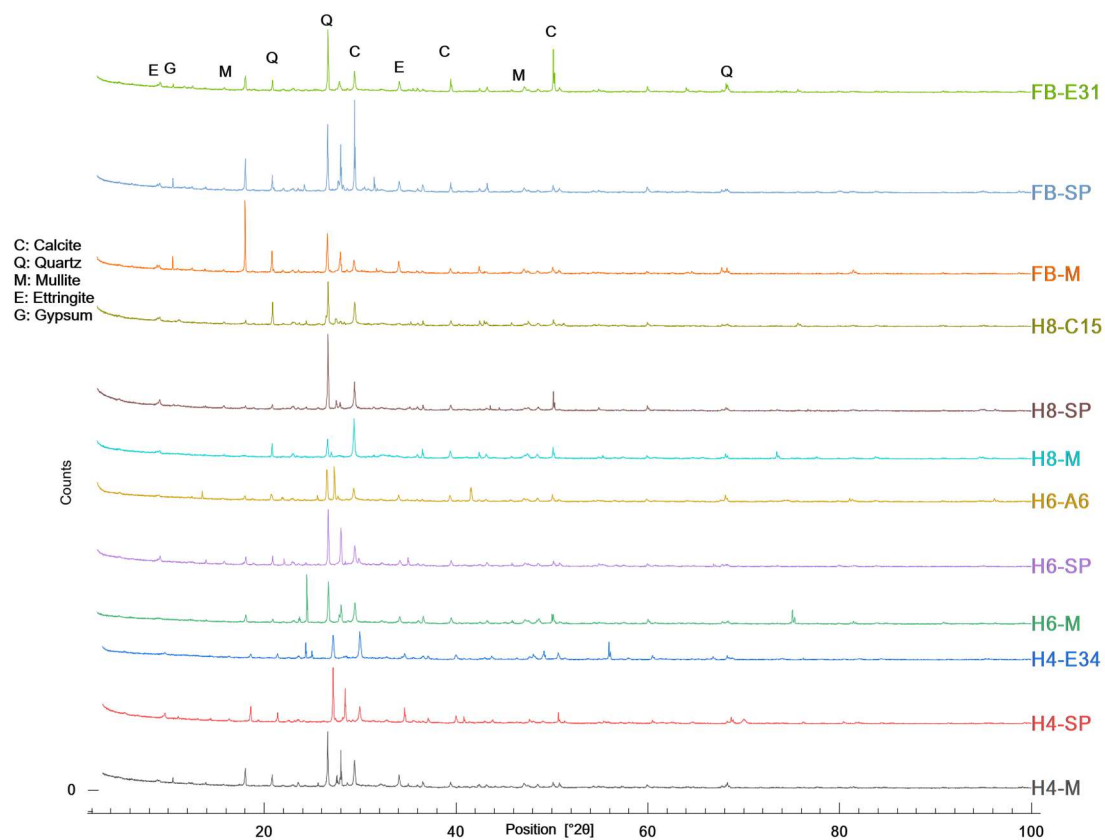


Figure 3. 23 XRD analysis of samples (control and with bacteria)

4 CONCLUSION

This study aimed to find bacterial alternatives to *S. pasteurii* bacteria, widely used in bacterial-healed concrete. Using five isolates of bacteria extracted from local caves in Türkiye, the efficiency of these five isolates was compared with *S. pasteurii* bacteria by exposing the samples to different factors that cause microcracks regardless of the application of healing and repair using bacteria. Through the results obtained from the tests and as a result of their study and analysis, it can be concluded that:

- The different tests showed variations in bacterial healing efficiency. At the same time, extensive recovery results were obtained in the samples exposed to high temperatures for all tests (Ft, FC, WA, CWA, and UPV) due to the small size of the cracks. On the contrary, in the samples that were cracked by flexural loads, the recovery of the flexural strength was relatively small due to the large cracks.
- The results generally showed high healing rates in some tests (WA, CWA, and UPV) while these rates were lower in the tests (Ft and Fc). This indicates the effectiveness of bacteria in filling the voids and improving the properties of the cementitious material for water absorption and its consequences, compared to improving the mechanical properties, which showed less improvement.
- Microstructural analysis of the samples confirmed the effectiveness of the bacterial isolates in precipitating calcium carbonate through the appearance of calcite deposits in the SEM analysis, an increase in calcium concentration in the EDS analysis, and the appearance of peaks of calcite positions in the XRD analysis.

- The isolates C15, E31, and D25 with a urease-negative approach showed better results than the SP bacteria with a urease-positive approach. Using urease-negative bacteria demonstrated a more sustainable approach to healing concrete by minimizing the potential environmental risks associated with ammonia production.
- The use of bacteria with properties capable of precipitating calcium carbonate leads to enhancing the physical and mechanical properties of the mortar in the bacterial healing process.
- Bacteria strains vary in efficiency under different test conditions; for example, E31 shows high efficiency in healing samples exposed to high temperatures, but its efficiency is lower in samples that have undergone freeze-thaw cycles.
- The statistical analysis showed that E31 and A6 bacteria are more efficient than SP bacteria in cement mortar healing, and therefore, they can be considered alternatives that can be used.
- It is recommended to use the isolates that showed high efficiency in more profound studies and more focused healing methods to study these isolates better, which contributes to benefiting from this study and enriching the literature on the use of bacteria in bacterial healing.

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PUBLICATIONS FROM THE THESIS

Conference Papers

1. Paper 1 : Comparison of Various Bacterial Species with *Sporosarcina Pasteurii* in Self-Healing Cement Mortar

https://kongre.akademikiletisim.com/files/icar14/icar14_ozet_kitapcigi.pdf

Papers

1. Paper 1: Comparison of Bacterial Healing Performances of Cave Bacteria and *Sporosarcina pasteurii* in Cement-Based Mortar

Processes **2025**, 13(3), 673; <https://doi.org/10.3390/pr13030673>