Study on Bioremediation of Heavy metals Present in Cement

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ABSTRACT
Cement, crucial for construction, poses significant environmental risks due to heavy metal contamination, including lead, cadmium, chromium, nickel, etc. These metals, released during cement production, endanger both human health and ecosystems. An innovative approach involves utilizing microorganisms for bioremediation, converting pollutants into less harmful forms. Microbes develop resistance mechanisms against heavy metals, reducing their concentration and mobility within cement. In this project, various brands and types of cement were collected, and different bacteria were cultured. A comparison was made regarding the mechanical properties, concentration of heavy metals, elemental composition, surface morphology, and particle size of the cement before and after bioremediation using Atomic Absorption Spectroscopy (AAS), Energy Dispersive X-ray Analysis (EDAX), and Scanning Electron Microscopy (SEM), respectively. The results obtained from the tests conducted were compared between the conventional and bacteria-induced cement samples. Implementation of such biotechnological approaches not only addresses environmental concerns but also promotes the development of innovative and sustainable solutions in construction.

Keywords: Cement, Heavy metals, Bioremediation, Microorganisms, Atomic Absorption Spectroscopy (AAS), Energy Dispersive X-ray Analysis (EDAX), Scanning Electron Microscopy (SEM), Sustainable.

INTRODUCTION

1.1 CEMENT

Cement stands as a pivotal binding agent in construction, renowned for its capacity to set, harden, and effectively adhere to other materials. Comprised mainly of calcium, silicon, aluminium, iron, and various other components, cement is available in diverse forms, ranging from the widely utilized Portland cement to specialized blends tailored to specific construction needs. However, cement production significantly contributes to global CO_2 emissions. The manufacturing process involves heating raw materials in a cement kiln, resulting in the release of CO_2 stored in calcium carbonate. Nonetheless, hydrated cement products, such as concrete, gradually reabsorb atmospheric CO_2 through the carbonation process, thereby mitigating initial emissions. Cement's essential characteristics profoundly influence the load-bearing capacity of structures, the setting time upon mixing with water, and the ease of handling and placement in construction field. Yet, the environmental impact of cement production has garnered considerable attention due to concerns surrounding carbon dioxide emissions and resource-intensive manufacturing practices. Furthermore, the presence of heavy metals in cement poses significant environmental challenges, emphasizing the urgent need for sustainable practices and innovative solutions.

Fig 1.1 Different brands of cement bags

1.2 HEAVY METALS

Heavy metals, characterized by their elevated atomic weights and densities, pose significant environmental and health concerns. These metals, including lead, mercury, cadmium, arsenic, and chromium, exhibit toxic properties with adverse effects on both human health and ecosystems. Their ability to accumulate in the food chain, coupled with their persistence in the environment, necessitates strict regulatory measures mitigate potential exposure. Remediation techniques, such as bioremediation, offer avenues for mitigating contamination, contribute to addressing the challenges associated with heavy metal pollution.

In the realm of construction, heavy metals find their way into cement, either as inherent components or introduced during the production process, with metals like lead, cadmium, and chromium being notable examples. This presence raises environmental concerns, regarding potential leaching into surrounding soil and water during the lifecycle of cement-based structures. Including the use of microorganisms for bioremediation to reduce heavy metal content in cement. The below mentioned table 1.1 illustrates the adverse health impacts of each heavy metal that require reduction.

Table.1.1 Toxic Effects of Heavy Metals on Human Health

S.no	Name of the heavy metal	Toxic effects on human health
1	Chromium	It has capacity to penetrate human skin causing chromium dermatitis called “cement itch”.
2	Lead	Liver damage, Abdominal pain, Diarrhea and Vomiting.
3	Nickel	Affects the Nervous, Digestive and Immune systems, Lungs and Kidneys.
4	Arsenic	Irritation to skin, Harms lungs, stomach and kidneys also leads to cancer.
5	Cadmium	Damages the Kidney, Respiratory and Skeletal system and also increases the blood pressure.
6	Mercury	Leads to cancer, Cardiovascular disease and Diabetes.

1.3 BIOREMDIATION

Bioremediation is an eco-friendly approach that utilizes living organisms, such as bacteria, fungi, and plants, to mitigate environmental pollution. This technique leverages the metabolic capabilities of these organisms to either degrade or immobilize pollutants, transforming them into less harmful forms. It offers a sustainable alternative to traditional remediation methods, reducing the environmental impact of pollution while promoting ecosystem restoration.

In the context of heavy metal contamination, bioremediation involves harnessing the unique abilities of microorganisms to reduce the concentration of metals in the environment. The application of bioremediation techniques to heavy metals, such as lead, cadmium, and chromium, has shown promise in mitigating the environmental and health risks associated with these pollutants. This environmentally conscious approach is particularly relevant in industries like construction, where heavy metals may be present in materials like cement. Research in this area focuses on optimizing bioremediation strategies for heavy metals, contributing to the development of sustainable and effective solutions for environmental cleanup.

1.4 MICRO ORGANISMS AND THEIR ROLE

Microorganisms, play fundamental roles in various ecological processes, from nutrient cycling to decomposition, highlighting their significance in maintaining environmental balance. In addition to their natural functions, microorganisms have gained attention for their applications in biotechnology, medicine, and environmental management, showcasing their adaptability and versatility across disciplines.

$$M^{2+} + CO3^{2-} \rightarrow MCO3$$

Various microorganisms play integral roles in the reduction of heavy metals, contributing to the process of bioremediation. Pseudomonas aeruginosa, renowned for its versatility, actively participates in reducing and detoxifying heavy metals such as nickel. Bacillus aluvi, another notable microorganism, is effective in the reduction of lead contamination by absorbing, accumulating, and precipitating lead ions, thus contributing to the remediation of lead-polluted environments. Actinobacteria, a diverse bacterial group, engages in reducing heavy metals like chromium through various metabolic pathways, rendering them less harmful. Shewanella oneidensis is recognized for its ability to reduce and precipitate metals like uranium, using electron transfer mechanisms to convert soluble uranium into insoluble forms.

1.4.1 CALCIUM CARBONATE PRECIPITATION

- When calcium ions (Ca^{2+}) and carbonate ions ($CO3^{2-}$) come together in a solution, they can react to form solid calcium carbonate.
- The chemical equation for this reaction is:

  $$Ca^{2+} + CO3^{2-} \rightarrow CaCO^{3}$$

- This process is known as precipitation because the calcium carbonate comes out of the solution and forms a solid, often seen as a white, chalky substance.

1.4.2 HEAVY METAL PRECIPITATION

- When heavy metal ions (M^{2+}) are present in a solution along with carbonate ions ($CO3^{2-}$), they can also und
- The general chemical equation for this reaction is:
- The specific metal carbonate formed depends on the type of heavy metal present. For example, if the heavy metal is lead (Pb), the resulting compound would be lead carbonate ($PbCO^{3}$).

In both cases (Calcium precipitation and metal precipitation), the carbonate ions act as a "glue" that binds withs the calcium ions or heavy metal ions, causing them to come out of solution and form solid carbonate compounds.

This precipitation process is essential for immobilizing heavy metals, reducing their mobility and potential harm to the environment.

Fig.1.2 Raoultella-ornithinolytica

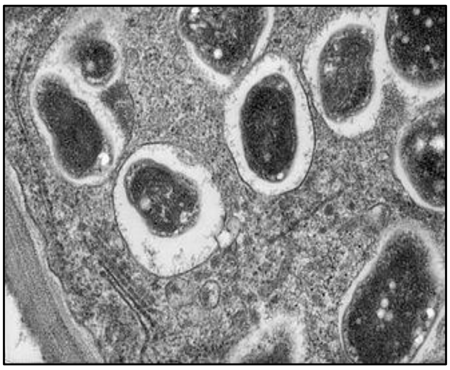


Fig.1.3 Bradyrhizobium

Fig.1.4 Pseudomonas putida

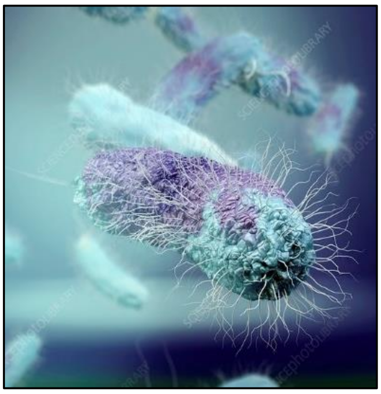


Fig.1.5 Bacillus pumilus

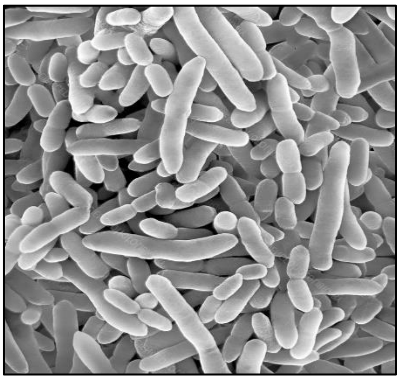


Fig.1.6 Rhodococcus

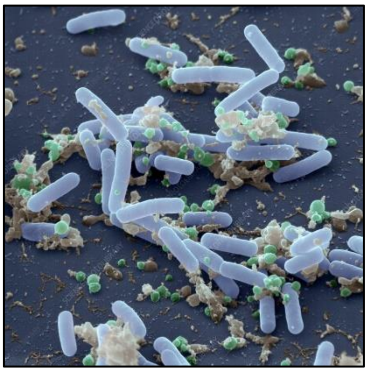


Fig.1.7 Shewanella onidensis

1.5 ATOMIC ABSORPTION SPECTROSCOPY

Atomic Absorption Spectroscopy (AAS) is a powerful analytical technique used to quantify the concentration of specific elements, particularly metals, in a sample. The method relies on the principle that atoms absorb light at characteristic wavelengths when subjected to a light source. In AAS, a sample is vaporized, and the resulting atomic vapor is exposed to a light beam. The absorption of light is then measured, allowing for the precise determination of the element's concentration in the sample.

AAS enables the identification and quantification of heavy metals, such as lead, cadmium, mercury, and chromium, in diverse matrices, including water, soil, and biological samples. The precise measurement capabilities of AAS contribute significantly to understanding and managing the presence of heavy metals, facilitating informed decision-making in environmental and public health contexts.

Fig.1.8 Atomic absorption spectroscopy

1.6 ENERGY DISPERSIVE X-RAY ANALYSIS (EDAX)

Energy Dispersive X-ray Analysis (EDAX), also known as Energy Dispersive Spectroscopy (EDS), is a powerful analytical technique used to determine the elemental composition of materials. It operates by bombarding a sample with high-energy electrons, which causes the emission of characteristic X-rays from the atoms in the sample. These emitted X-rays are then collected and analyzed to identify the elements present in the sample and quantify their concentrations.

Fig.1.9 Energy dispersive x-ray analysis (EDAX)

This meticulous analysis offers invaluable insights into the alterations taking place within the cement matrix due to microbial activity. Moreover, EDAX enables us to identify any potential changes in the chemical structure of the cement, shedding light on the mechanisms underlying microbial interactions with heavy metals.

1.7 SCANNING ELECTRON MICROSCOPY (SEM)

Scanning Electron Microscopy (SEM) provides a valuable glimpse into the intricate world of the microscopic domain. It allows for detailed examination of the surface morphology, texture, and particle size of cement specimens with exceptional resolution. Through SEM analysis, researchers can delve into the microstructural features of cement materials, unraveling critical insights into their physical properties and composition.

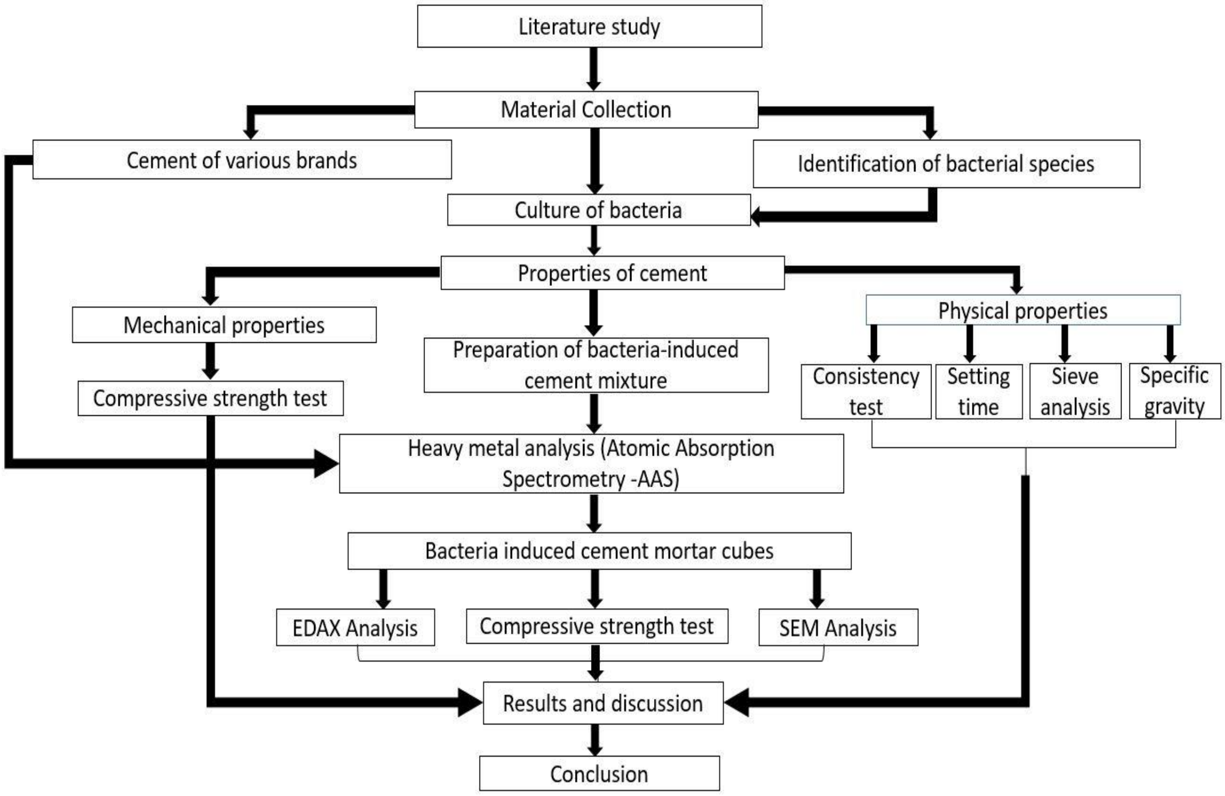
This comprehensive analysis not only facilitates a deeper understanding of the inherent characteristics of cement but also sheds light on the efficacy of bioremediation processes. By scrutinizing the interactions between microorganisms, cement, and heavy metal pollutants at the microscopic level, SEM offers invaluable insights into the complex dynamics at play during remediation efforts.

Fig.1.10 Scanning electron microscopy (SEM)

1.7 OBJECTIVE

- To determine the concentration of heavy metals in cements from various brands using AAS.
- To evaluate the physical and mechanical properties of cement.
- To culture various bacteria.
- To analyze the reduction in concentration of heavy metals in cement using AAS after the bioremediation process.
- To prepare bacteria induced cement mortar cubes.
- To compare the compressive strength before and after bioremediation.
- To examine the elemental composition and interactions between the bacteria and cement before and after the bioremediation using Energy Dispersive X-ray Analysis (EDAX) and Scanning Electron Microscopy (SEM) respectively.

1.8 METHODOLOGY



LITERATURE REVIEW

2.1 LITERATURE REVIEW

Roohallah Saberi Riseh ,Mozhgan Gholizadeh Vazvani (2023) explored the heavy metal accumulation in soils affects plant performance, morphology, and physiology. Heavy metals have irreversible effects on the environment. Plant Growth-Promoting Rhizobacteria (PGPRs) can mediate interactions between plants and heavy elements. Isolating metal-resistant bacteria from polluted sites offers promising bioremediation solutions.

Subitha Thirupathi, Manju Rajamani (2023) reported that the various organisms effectively reduced heavy metal toxicity in cement. Bacillus family bacteria showed significant toxicity reduction, with Bacillus megaterium (Mg) and Bacillus licheniformis (Fe) leading by 42% and 20%. Isolated strains, including Bacillus subtilis (Cu), Pseudomonas otitidis (Pb), and Pseudomonas aeruginosa (Ni), achieved notable reductions of 10%, 13%, and 15%, respectively.

Abateayele, Yakob Godebo (2021) focused on microorganisms like bacteria, fungi, and microalgae offer a sustainable solution for reducing excess Chromium (VI) levels in the environment. Biosorption, a cost-effective technology, involves transport across cell membranes, complexation, ion exchange, and precipitation. It plays a vital role in removing chromium from water.

Eswar Sairam Ravipati, Nikhil Nitin Mahajan (2021) examined that heavy metals like lead pose significant toxicity risks to humans, animals, and plants. Electrochemical approaches are sensitive but lack selectivity for specific ions like lead. This review provides insights into lead's regulatory, toxicity, and analytical aspects.

Fatini Mat Arisah, Amirah Farhana Amir (2021) demonstrated that Pseudomonas aeruginosa RW 9 effective removal of up to 85% of 10 mg/L chromium (VI). Extracellular sequestration was the primary mechanism for chromium (VI) removal, accounting for over 50% of total removal of chromium (VI) induced the synthesis of biosurfactants, identified as rhamnolipids.
Xiaoxia Yu, Jintong Zhao (2021) discussed about soil ecosystems rely on microbes for various processes, especially in heavy metal-contaminated environments. Cadmium-tolerant phyla like Proteobacteria and Gemmatimonas play vital roles, promoting ecosystem resilience in the presence of heavy metals.
Mahendra Aryal (2020) investigated Bacterial bio sorption is effective for removing heavy metals (HMs) from contaminated sites. Operating conditions such as pH, biomass concentration, contact time, temperature, and initial metal concentrations influence bio sorption performance. Bacterial biomass shows superior performance compared to other biomaterials.
Prakash Mallappa Munnoli, Sudisha Jogayya (2020) experimented that A 40% suspension exhibited higher compressive strength compared to 20% and 60% in all cases B. Subtilis with optimized 40% suspension (CFU 10x10^8/ml) showed increases in CS of 4.32%, 5.56%, and 3.81% for 3 days, 7 days, and 28 days respectively. Overall, B. Subtilis resulted in a 5.92% increase in CS compared to the control cube at 3 days.
Ya-Nan Xu, Yinguang Chen (2020) demonstrated the enhanced sulfate reduction focus on improving sulfate reduction for efficient heavy metal removal by sulfate-reducing bacteria (SRB). The heavy metal is removed by immobilizing SRB, creating a protective barrier between them and the harmful metals. When different types of bacteria work together, they can be more efficient. Some bacteria can even soak up heavy metals.
Yogesh Jayant, Saresh K Jain (2020) investigated that Bacteria aid healing by producing calcite, improving compressive strength, durability, and reducing water permeability. Maintain low bacterial concentration and use 0.50% calcium lactate for effective results.
N.K. Srivastava, C.B. Majumdernove (2019) focused on the advances in microbial cloning techniques can enhance removal efficiency, reducing treatment costs. Biofilters can remove heavy metals down to ppb levels, making them cost-effective for industries like chemicals, fertilizers, textiles, and more.
Hitendra Shivhare, Prof. Vijay Kumar Shrivastava (2018) examined that the Concrete containing 100% bacteria solution demonstrated a compressive strength of 23.98 N/mm2 after 7 days, while concrete with 70% bacteria solution exhibited a strength of 33.95 N/mm2 after 28 days, both surpassing conventional concrete.Inclusion of bacteria increased concrete compressive strength, mainly due to microbiologically induced calcium carbonate precipitation (MICCP), filling pores within concrete cubes.
Joseph Thatheyus, D. Ramya (2016) studied that wastewater treatment, involving methods like chemical precipitation. The traditional high-pH approach generates waste and is less effective at low chromium (VI) concentrations. Bacteria, such as Pseudomonas and Bacillus, detoxify chromium by reducing chromium (VI) to chromium (III) with diverse resistance mechanisms. They show promise for environmental clean-up.
Wasiatus Sa’diyah, Endang Suarsini (2016) concluded that bacterial consortium (B. alvei and B. pumilus) achieves 93.58% lead reduction at a 7% culture concentration, meeting quality standards. The consortium's success arises from the synergistic action of B. alvei and B. pumilus, enabling higher lead reduction.
Aparna K Sathyan (2015) concluded the Mortar cube strength increases up to 10^7 cells/ml bacteria concentration but decreases thereafter. Optimal bacteria doses boost compressive strength by 58% (7 days) and 23% (28 days) over controls.SEM analysis confirms bacterial involvement in calcite production.

A. Singh, S. M. Prasad (2015) carried out the growing awareness of pollution motivates the development of clean-up technologies, particularly for heavy metal contamination. Promoting eco-friendly solutions is essential in addressing heavy metal contamination. To combat heavy metal pollution, various techniques are used, including low-cost absorbents, chelating agents, phytoremediation, and even molecular and nanotechnology approaches

Mireille Bruschi, Larry L. Barton (2015) reported the mechanisms behind how sulfate-reducing bacteria detoxify toxic metals like mercury, chromate, and arsenate are not entirely clear. Sulfate-reducing bacteria provide an eco-friendly option for metal detoxification. They can be used for soil and groundwater treatment, and their enzymes can create biosensors to measure metal bioavailability.

Paul B. Tchounwou, Clement G. Yedjou (2012) investigated heavy metals like arsenic, cadmium, chromium, lead, and mercury exist naturally, but human activities significantly contaminate the environment with them. It's vital to understand how these metals interact on a molecular level and health management when dealing with mixtures of toxic elements.

Pratik M. Choksia, Vishal Y. Joshib (2007) studied that natural adsorbent materials, without activation, Or nickel (II) and aluminium (III) ions, the ideal pH is 7.5 and 6.5, respectively. Clay (bleaching earth) is particularly effective for removing these ions. The pseudo-first-order chemical reaction model provides the best fit for the data.

2.2 OBSERVATIONS FROM THE LITERATURE STUDY

Table.2.1 Bacteria that contributes in the reduction of heavy metals

S.no	Name of The Heavy Metal	Name of The Bacteria
1	Chromium	Pseudomonas aeruginosa, Pseudomonas putida.
2	Lead	Sulphate reducing bacteria, Desulfovibrio alaskenis 6sR, Bacillus aluvi, Bacillus pumilus.
3	Mercury	Bacillus species, Sulphate reducing bacteria.
4	Arsenic	Sulphate reducing bacteria, Pseudomonas aeruginosa, shewanella onidensis, Pesulfito bacterium
5	Cadmium	Rhizo bacterium, Bacillus species, Baerkholderia species, micro bacterium oxydens CM3, Rhodococcus species,
6	nickel	Paracoccus dentrificans, Actino bacteria, Corynebacterium, Rhodococccus.

- The table 2.1 illustrates the observed contributions to the reduction of heavy metals found in the literature.
- Bioremediation processes, which include the utilization of bacteria to decrease heavy metal levels, have the potential to alter certain properties of soil and water bodies. These changes can affect soil pH, texture and structure, nutrient content, and overall water quality.
- Bacillus megaterium and Bacillus licheniformis from the Bacillus family reduced toxicity by 42% and 20% respectively.
- Pseudomonas aeruginosa (Ni) also achieved a notable reduction of 15%.
- Desulfovibrio alaskensis strain 6SR showed a remarkable ability to remove 98% of lead in solution.
- Paracoccus denitrificans AC-3 was able to remove 46.19% of nickel.

- The bio sorption capacity of Pseudomonas aeruginosa is 98%, and Pseudomonas aeruginosa RW 9 demonstrated effective removal of up to 85% Chromium.
- Based on various literature, it has been observed that bioremediation is a cost-effective and eco-friendly alternative to physicochemical methods.
- Many studies have implemented bioremediation using bacteria such as Pseudomonas species, Bacillus species, Rhizobacterium, etc., which have demonstrated effective results in reducing metals like chromium, lead, and cadmium present in soil and water bodies. Therefore, the application of these bacteria in cement could potentially yield positive outcomes.
- The bacteria have been observed to not only reduce the concentration of heavy metals but also alter the properties of soil or water bodies. Consequently, the introduction of these bacteria into concrete could potentially modify its properties as well.
- Biosorption, a cost-effective technology, involves transport across cell membranes, complexation, ion exchange, and precipitation, playing a vital role in chromium removal from water.
- Factors such as pH levels, biomass density, and duration of contact, temperature, and initial metal levels directly impact the effectiveness of biosorption.
- B. Subtilis resulted in a 5.92% increase in Compressive strength compared to the control cube at 3 days.
- Bacterial consortium (B. alvei and B. pumilus) achieves 93.58% Pb reduction at 7% culture concentration, meeting standards.
- Inclusion of bacteria increased concrete compressive strength, mainly due to microbiologically induced calcium carbonate precipitation (MICCP), filling pores within concrete cubes.
- Concrete containing 100% bacteria solution demonstrated a compressive strength of 23.98 N/mm2 after 7 days, surpassing conventional concrete.

MATERIALS

3.1. CEMENT SAMPLES

Cement samples from four distinct brands, encompassing both 53-grade Ordinary Portland Cement (OPC) Confirming to IS: 2269-1987 and Portland Pozzolana Cement (PPC) Confirming to IS: 1489-1(1991), were systematically gathered for this project. In total, eight cement samples were collected, and the table3.1 below shows the detailed list is provided

Table.3.1 Details of obtained cement samples

S.no	Name of the brand	Grade of the cement	Type of the cement
1	Birla shakti	-	PPC
2	Chettinadu	-	PPC
3	Coromandel	-	PPC
4	Dalmia	53	OPC & PPC
5	Maha	53	OPC & PPC
6	Ramco	53	OPC & PPC
7	Ultratech	53	OPC & PPC

Fig.3.1 Dalmia - OPC and PPC

Fig.3.2 Ramco - OPC and PPC

Fig.3.3 Maha - OPC and PPC

Fig.3.4 Ultratech - OPC and PPC

Fig.3.5 Chettinadu PPC

Fig.3.6 Birla Shakti PPC

Fig.3.7 Coromandel PPC

3.2 FINE AGGREGATE

Fine aggregate confirming to IS: 383-1970, a fundamental component in concrete mixtures, consists of small, granular particles such as sand. Its primary role is to fill the voids between coarse aggregates and bind together the cement paste, contributing to the overall strength and durability of the concrete. The quality and grading of fine aggregate significantly influence the workability, cohesion, and finish of the concrete mix. The appropriate selection and proportioning of fine aggregate play a crucial role in achieving the desired properties of the concrete, making it a key consideration in construction practices.

Fig.3.8 M-sand

3.3 BACTERIA

Pseudomonas aeruginosa, Bacillus aluvi, and Actinobacteria are noteworthy bacteria with unique capabilities in environmental processes. Pseudomonas aeruginosa plays a vital role in reducing nickel contamination, showcasing its potential in bioremediation efforts. Bacillus aluvi is recognized for its contribution to the reduction of lead, demonstrating its efficacy in mitigating the impact of this heavy metal. Actinobacteria, with its distinctive characteristics, actively contributes to the reduction of chromium, further highlighting the diverse and beneficial roles that bacteria play in addressing environmental challenges associated with heavy metal pollutants.

3.3.1 CULTIVATION OF BACTERIA

- The cultivation of bacteria involves a series of precise steps to create a conducive environment for their growth.
- Initially, in a conical flask, 2.9 grams of nutrient broth are meticulously mixed with 100 ml of distilled water, forming a nutrient-rich medium essential for bacterial proliferation.
- The conical flask is then carefully sealed with cotton and subjected to sterilization in an autoclave at a temperature of 121^{o} C, ensuring the elimination of any potential contaminants.
- After the sterilization process, the prepared sample is inoculated with 1 ml of bacteria under the controlled conditions of a laminar airflow chamber.
- This step is crucial to introduce a controlled amount of bacterial culture into the nutrient broth, initiating the growth process.
- The inoculated culture is then transferred to an incubator set at a specific temperature, in this case, 35 degrees Celsius, for a defined period, typically 24 hours.
- The incubation period allows for the optimal conditions needed for bacterial replication and the formation of a robust culture.

Fig.3.9 Nutrient broth

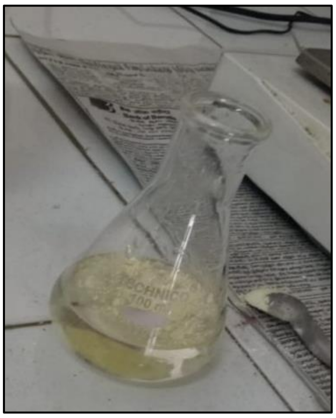


Fig.3.10 Nutrient broth mixed with distilled water

Fig.3.11 Autoclave for Sterilization

Fig.3.12 Inoculation process of bacteria

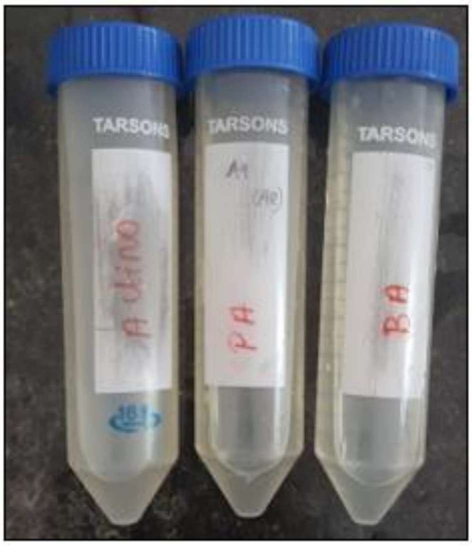


Fig.3.13 Actino bacteria, Pseudomonas aeruginosa, Bacillus aluvi (Bacterial cultures)

EXPERIMENTAL INVESTIGATION

4.1 TEST CONDUCTED ON CEMENT

The examination of the cement involved conducting tests to evaluate its mechanical and physical properties such as,

- The initial and final setting time of cement
- The standard consistency test
- Dry sieving method
- Specific gravity
- Compressive strength test

Additionally, Atomic Absorption Spectroscopy (AAS) analyses were carried out to further characterize the presence of heavy metals in cement.

4.1.1 INTIAL AND FINAL SETTING TIME OF CEMENT (As per IS 4031 Part-5-1988)

The initial setting time of cement refers to the duration it takes for the cement paste to change from a plastic to a rigid state after the addition of water. It indicates the timeframe available for the handling, mixing, and placing of cement in construction before it begins to set.

The final setting time is the point at which the cement paste attains complete. It establishes the timeframe within which the cement achieves its ultimate strength and stability, marking the end of the setting process.

4.1.1.1 PROCEDURE

- **Initial Preparation:**

1. Conduct a consistency test to determine the water needed for normal paste consistency (P).
2. Mix 400 g of cement with 0.85P water, ensuring a gauge time of 3 to 5 minutes.
3. Record the time (T1) when water is added and stopwatch started.
4. Fill the Vicat mould with the prepared cement paste to create a test block.

- **Test for Initial Setting Time:**
1. Place the test block under the needle-bearing rod and quickly release the needle at 2-minute intervals.
2. Record the time (T2) when the needle fails to pierce the block by about 5 mm.
- **Test for Final Setting Time:**
1. Replace the needle with an annular attachment on the Vicat apparatus.
2. Cement is considered finally set when the final setting needle makes an impression on the test block, while the attachment fails to do so.
3. Record the time (T3) as the final setting time.

Table.4.1 Test results of initial and final testing time

S.no	Cement brand	Grade and type of cement	Initial setting time (minutes)	Final setting time (minutes)
1	Birla shakti	PPC	30	270
2	Chettinadu	PPC	30	300
3	Coromandel	PPC	45	240
4	Dalmia	PPC	45	330
5	Maha	PPC	35	300
6	Ramco	PPC	35	270
7	Ultratech	PPC	50	300
8	Dalmia	53 & OPC	35	240
9	Maha	53 & OPC	30	300
10	Ramco	53 & OPC	45	270
11	Ultratech	53 & OPC	45	300

The above table 4.1 shows the results of initial and final setting time of cement. Hence the minimum Initial and maximum Final setting time of a cement should be 30 minutes and 600 minutes respectively as per **IS 269:2015 and IS 1489: Part 1:2015.**

4.1.2 STANDARD CONSISTENCY TEST (As per IS 4031 Part 4: 1988)

The standard consistency test assesses the optimal water content for normal consistency in cement. It determines the right balance between fluidity and cohesion in cement paste, crucial for quality control in construction. Achieving standard consistency ensures optimal performance in various applications, guiding water-to-cement ratios for effective concrete mixes.

4.1.2.1 PROCEDURE

1. Weigh 400 grams of cement and mix it gradually with a calculated amount of water until a uniform paste is achieved.
2. Transfer the cement paste into the Vicat mould, ensuring thorough filling without voids.
3. Lower the Vicat plunger gently onto the paste surface and release it quickly, repeating until no impression is left.
4. The consistency is considered standard when the Vicat plunger penetrates the paste to a depth of 10 to 12 mm under a 50 N load.
5. Record the water amount used and the achieved consistency, providing crucial information for quality control and further assessments.

Fig.4.1 Vicat apparatus

Fig.4.2 400g of Cement

Fig.4.3 Mixing of cement and water

Table.4.2 Test results of Standard consistency test

S.no	Name of the brand	Grade and type of cement	Percentage of water content
1	Birla shakti	PPC	35 %
2	Chettinadu	PPC	35 %
3	Coromandel	PPC	32 %
4	Dalmia	PPC	33 %
5	Maha	PPC	32 %
6	Ramco	PPC	34 %
7	Ultratech	PPC	33 %
8	Dalmia	53 & OPC	28 %
9	Maha	53 & OPC	32 %
10	Ramco	53 & OPC	33 %
11	Ultratech	53 & OPC	30 %

The table 4.2 represents the results of standard consistency test on cement. Hence the percentage of water content of a cement of grade 53 & OPC and PPC should be 25-35% and 32% - 36% respectively as per **IS 269:2015** and **IS 1489: Part 1:2015.**

4.1.3 DRY SEIVING METHOD (As per IS 4031 part 1: 1996)

The fineness of cement refers to the particle size distribution and surface area of cement particles. It is a crucial property that influences the hydration and setting characteristics of cement, as well as the strength and durability of concrete produced with it. Finer cement particles provide a larger surface area for hydration reactions to occur, leading to faster setting times and increased early strength development. The fineness of cement is typically measured by specific surface area, which is determined through methods such as the Blaine air permeability test or the laser diffraction technique. A higher specific surface area indicates finer cement particles, while a lower specific surface area indicates coarser particles. The fineness of cement is an important quality control parameter in cement production to ensure consistent and optimal performance in concrete applications.

4.1.3.1 PROCEDURE

1. Take 1000 grams (1 Kg) of cement for the test sample and name it as (w1).
2. Rub the cement particle well with your hands so that no lumps are left.
3. Pour the 1 Kg cement content in the sieve and close it perfectly with the sieve lid.
4. Put the sieve in the shaking machine and start the machine for 15 minutes.
5. Brush the sieve base gently with the bristle brush so that nothing is left on the sieve surface.
6. Weigh the retained amount of cement on the sieve and note it as (w2).
7. Find the percentage of the weight of cement-retained on the 90 µm sieve.

Fig.4.4 90 microns sieve and a pan

Fig.4.5 100 grams of cement sample

Fig.4.6 Retained residue of the cement sample weighed

S.no	Name of the brand	Grade and type of cement	Percentage of fineness
1	Birla shakti	PPC	4.6 %
2	Chettinadu	PPC	4.0 %
3	Coromandel	PPC	4.8 %
4	Dalmia	PPC	5.6 %
5	Maha	PPC	5.0 %
6	Ramco	PPC	5.8 %
7	Ultratech	PPC	4.4 %
8	Dalmia	53 & OPC	5.2 %
9	Maha	53 & OPC	5.5 %
10	Ramco	53 & OPC	6.0 %
11	Ultratech	53 & OPC	4.6 %

The table 4.3 represents the results of fineness of cement. Hence the percentage of of fineness of cement of grade 53 & OPC and PPC should be less than 10 % as per

IS 4031 part 1: 1996.

4.1.4 SPECIFIC GRAVITY OF CEMENT (As per IS 4031 PART-11 1988)

Specific gravity of cement refers to the ratio of the mass of a given volume of cement to the mass of an equal volume of water at a specified temperature. It is a fundamental property that helps assess the density or compactness of cement particles. Specific gravity is an important parameter in determining the quality and consistency of cement, as it can influence various properties of concrete, including its strength, durability, and workability. A higher specific gravity indicates denser cement particles, while a lower specific gravity suggests lighter particles.

4.1.4.1 PROCEDURE

1. The flask is allowed to dry completely and made free from liquid and moisture and the weight of the empty flask is taken as W1.
2. The bottle is filled with cement to its half (Around 50gm of cement) and closed with a stopper and it is weighed with stopper and taken as W2.
3. To this kerosene is added to the top of the bottle. The mixture is mixed thoroughly and air bubbles are removed. The flask with kerosene, cement with stopper is weighed and taken as W3.
4. Next, the flask is emptied and filled with kerosene to the top. The arrangement is weighed and taken as W4.
5. Specific Gravity of Cement is given by the formula,

$$S_g = \frac{W2 - W1}{(W2 - W1) - (W3 - W4) \times 0.79}$$

Fig.4.7 Empty flask (W1)

Fig.4.8 1/3 of cement + Flask (W2)

Fig.4.9 Cement + Kerosene + flask (W3)

Fig.4.10 Flask + Kerosene (W4)

Table.4.4 Test results of Specific gravity of cement

S.no	Name of the brand	Grade and type of cement	Specific gravity of cement
1	Birla shakti	PPC	3.15
2	Chettinadu	PPC	3.15
3	Coromandel	PPC	3.13
4	Dalmia	PPC	3.16
5	Maha	PPC	3.14
6	Ramco	PPC	3.14
7	Ultratech	PPC	3.12

8	Dalmia	53 & OPC	3.15
9	Maha	53 & OPC	3.15
10	Ramco	53 & OPC	3.15
11	Ultratech	53 & OPC	3.11

The table 4.4 represents the results of specific gravity of cement. Hence the specific gravity of cement of grade 53 & OPC and PPC should be between 3.1 and 3.15 as per **IS 4031 part 11: 1998**.

4.1.5 COMPRESSIVE STRENGTH TEST (As per IS 4031 Part 6: 1988)

The compressive strength test on cement mortar cubes is a pivotal assessment, gauging the material's ability to withstand axial loads and compression. This test provides essential information on the mortar's structural performance, influencing decisions regarding its suitability for construction applications. The measured compressive strength signifies the maximum stress the mortar can endure under uniaxial compressive loads, serving as a critical parameter for ensuring the durability and structural integrity of constructed elements.

4.1.5.1 PROCEDURE

1. Use a cement-to-sand ratio of 1:3 for the test.
2. Take 200 gm of cement, and mix it with 600 gm of sand for 1 minute.
3. Calculate the water needed for standard consistency using the formula, with P set to 30 cement,
4. Add water to the mixture and mix for three minutes.
5. Assemble the cube mould on a vibrating machine, applying mould oil before pouring in the mortar.
6. Vibrate at a rate of 12000 ± 400 per minute for 2 minutes. Remove the mould, level the top surface with a trowel, and repeat for other cubes.
7. After 24 hours, demould the cubes, mark with date and number, and submerge in a freshwater tank for curing.
8. Test three cubes each on the third and 28th days for compressive strength.
9. Measure cube weight and record data. Place cubes in a compression testing machine and apply a load at a rate of 35 N/mm²/mi. Note the load at which the cube is crushed.

4.1.5.2 QUANTITY CALCULATION

Six cubes were casted from each collected cement sample for the compressive strength test, which was conducted on the 7th and 28th days.

For 1 sample:

- Number of cubes = 6 Nos
- Cement = 6*200 = 1200 grams
- Sand = 6*600 = 3600 grams

Fig.4.11 Mould of size 70.6mm*70.6mm*70.6mm

Fig.4.12 Mixing of cement and sand in a tray

Fig.4.13 Cement mortar cubes

Fig.4.14 Demoulded cement mortar cubes

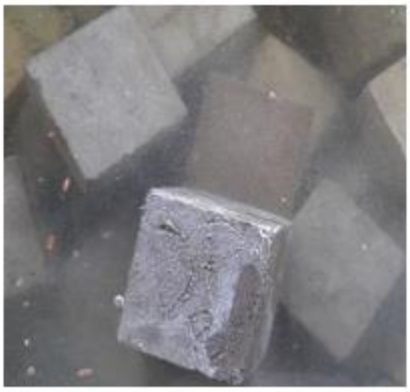


Fig.4.15 Curing of cement mortar cubes

Fig.4.16 Specimen before Loading

Fig.4.17 Specimen after loading

Table.4.5 Test results of Compressive strength test

S.no	Cement brand	Grade and type of cement	Compressive strength			
			7th day (N/mm²)		28th day (N/mm²)	
			Individual Cube	Average	Individual Cube	Average
1	Brila shakti	PPC	26.4	28.8	45.6	47.3
			29.8		50.3	
			30.4		46.2	
2	Chettinadu	PPC	28	27.2	45.4	46
			27.8		46.4	
			25.8		46.2	
3	Coromandel	PPC	27.2	26.2	51.3	51.4
			25		52.4	
			26.4		50.6	
4	Dalmia	PPC	26.1	24.8	40.6	43.7
			24.3		43.2	
			24.1		47.4	
5	Maha	PPC	24.08	25.96	47.4	46.6
			27		47.2	

			26.8		45.4	
6	Ramco	PPC	27	27.4	48.4	48.2
			27.4		47.6	
			28		48.6	
7	Ultratech	PPC	27	26.36	40.8	42.7
			25		44.6	
			27.08		42.8	

S.no	**Cement brand**	**Grade and type of cement**	**Compressive strength**			
			7th day (N/mm²)		**28th day (N/mm²)**	
			Individual Cube	**Average**	**Individual Cube**	**Average**
8	Dalmia	53& OPC	27.4	25.1	45.8	45.8
			24.5		45.2	
			23.4		46.4	
9	Maha	53& OPC	26.08	27.82	47.4	46.6
			29		47.2	
			28.4		45.4	
10	Ramco	53& OPC	24.1	23	47.2	48.8
			23		49	
			22.1		50.4	
11	Ultratech	53& OPC	26	25.8	42.8	43.6
			26.4		42.4	
			25.2		45.6	

The above table 4.5 represents the results of compressive strength for cement mortar cubes from eleven distinct cement samples, along with their average outcomes.

4.2 TEST CONDUCTED ON BACTERIA

4.2.1 COLONY FORMING UNITS

Colony-forming units (CFUs) of bacteria are viable bacterial cells capable of reproducing and forming visible colonies on a solid growth medium under specific conditions, used to estimate bacterial numbers in samples.

4.2.1.1 PROCEDURE

1. Prepare the agar medium by sterilizing by autoclave.
2. Pour the prepared agar into Petri dishes.
3. Prepare a series of dilutions by transferring specific volumes of the sample into sterile dilution blanks or test tubes containing sterile saline or distilled water.

4. Mix well after each dilution.
5. Spread the inoculum evenly over the surface of the agar using a sterile spreader or by rotating the plate gently.
6. Incubate the plates for 24 hours, depending on the growth characteristics of the bacteria at the temperature of 37°C.
7. Count the colonies on each plate and record the number of colonies for each dilution.

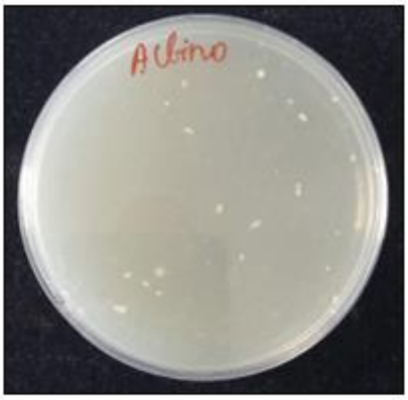


Fig.4.18 CFU Actino bacteria

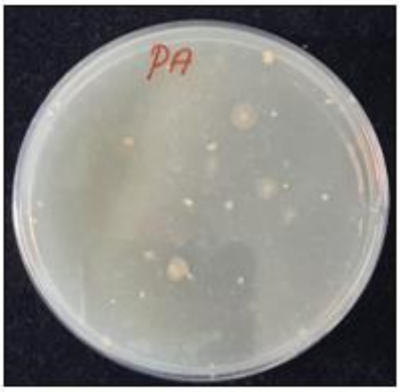


Fig.4.19 CFU Pseudomonas aeurginosa

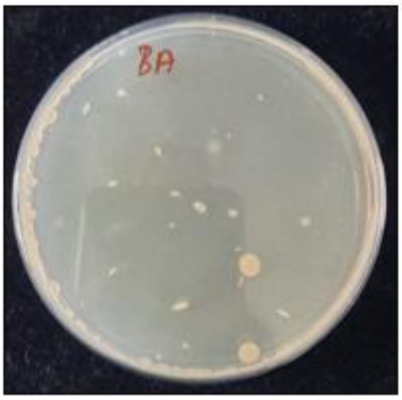


Fig.4.20 CFU Bacillus alluvi

COMMUNITY COMPOSITION ANALYSIS

A compatibility test of bacteria, often referred to as bacterial compatibility testing or bacterial susceptibility testing, is a method used to determine the sensitivity of bacteria to specific antimicrobial agents. This test involves exposing bacterial isolates to different antibiotics or antimicrobial compounds to assess their effectiveness in inhibiting bacterial growth or killing the bacteria.

4.2.2.1 PROCEDURE

1. Prepare the agar medium of three bacteria's namely pseudomonas aeurginosa, bacillus alluvi and action bacteria.
2. Pour the melted agar into sterile Petri dishes and allow it to solidify.
3. Spread the inoculum evenly over the surface of the agar using a sterile spreader or by rotating the plate gently.

4. Incubate the plates for 24 hours, depending on the growth characteristics of the bacteria at the temperature of 37°C.
5. Assess the growth of all bacterial strains. If there are no issues with their growth, we can utilize the three bacteria together. Otherwise, they cannot be used together.

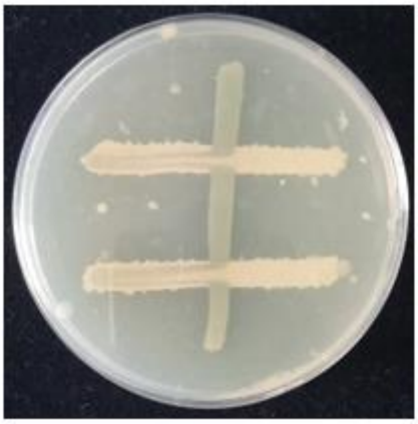


Fig.4.21 Community composition analysis of bacteria

4.3 TEST CONDUCTED ON BACTERIA INDUCED CEMENT MORTAR CUBES

The examination of the cement involved conducting tests to evaluate its mechanical and physical properties such as, Compressive strength test.

Additionally, Atomic Absorption Spectroscopy (AAS) analyses were carried out to further characterize the presence of heavy metals in cement.

4.3.1 COMPRESSIVE STRENGTH TEST (As per IS 4031 Part 6: 1988)

The compressive strength test on cement mortar cubes is a pivotal assessment, gauging the material's ability to withstand axial loads and compression. This test provides essential information on the mortar's structural performance, influencing decisions regarding its suitability for construction applications. The measured compressive strength signifies the maximum stress the mortar can endure under uniaxial compressive loads, serving as a critical parameter for ensuring the durability and structural integrity of constructed elements.

4.3.1.1 PROCEDURE

1. Use a cement-to-sand ratio of 1:3 for the test.
2. Take 200 gm of cement, and mix it with 600 gm of sand for 1 minute.
3. Calculate the water needed for standard consistency using the formula, with P set to 30 cement.
4. All the bacteria of 10ml, is also combined with the calculated amount of water.
5. Add water to the mixture and mix for three minutes.
6. Assemble the cube mould on a vibrating machine, applying mould oil before pouring in the mortar.
7. Vibrate at a rate of 12000 ± 400 per minute for 2 minutes. Remove the mould, level the top surface with a trowel, and repeat for other cubes.
8. After 24 hours, demould the cubes, mark with date and number, and submerge in a freshwater tank for curing.
9. Test three cubes each on the third and 28th days for compressive strength.
10. Measure cube weight and record data. Place cubes in a compression testing machine and apply a load at a rate of 35 N/mm²/mi. Note the load at which the cube is crushed.

Fig.4.22 Mixing of cement, sand, water+bacteria in a tray

Fig.4.23 Casted cement mortar cubes

Fig.4.24 Demoulded cement mortar cubes

Fig.4.25 Curing of cement mortar cubes

Table.4.6 Test results of Compressive strength test (bacteria induced cement)

S.no	Cement brand	Grade and type of cement	Compressive strength			
			7th day (N/mm²)		28th day (N/mm²)	
			Individual Cube	Average	Individual Cube	Average
1	Brila shakti	PPC	30.6	30.1	46.4	45.6
			29.6		46.8	
			30.2		46.2	
2	Chettinadu	PPC	30.8	30.2	50.2	50.2
			30.2		49.8	
			29.2		50.4	
3	Coromandel	PPC	30.6	30.8	45.8	45.06
			31.4		44.8	
			30.4		44.6	
4	Dalmia	PPC	30.8	30.06	47.8	46.9
			30.2		46.8	
			29.2		46.2	
5	Maha	PPC	29.8	29.4	49.8	50.13
			29.6		50.8	
			28.8		49.8	
6	Ramco	PPC	29.8	29.86	50.8	50.3
			29.6		49.8	
			30.2		50.4	
7	Ultratech	PPC	31.8	31.06	45.4	45.8
			30.8		45.8	

			30.6		46.2	

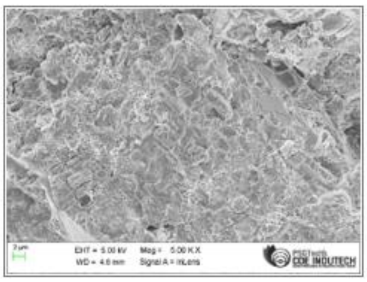
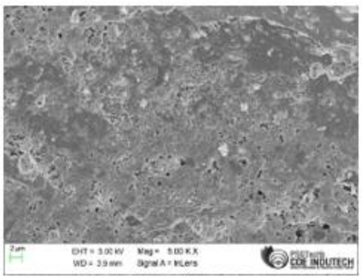
S.no	Cement brand	Grade and type of cement	Compressive strength			
			7th day (N/mm²)		28th day (N/mm²)	
			Individual Cube	Average	Individual Cube	Average
8	Dalmia	53& OPC	31.8	31	48.8	47.86
			30.4		47.6	
			30.8		47.2	
9	Maha	53& OPC	29.8	29.8	48.4	48.9
			29.2		48.8	
			30.6		49.6	
10	Ramco	53& OPC	28.6	29	51.4	51.8
			28.8		52.4	
			29.6		51.6	
11	Ultratech	53& OPC	30.2	29.8	52.8	52.4
			29.8		51.8	
			29.4		52.4	

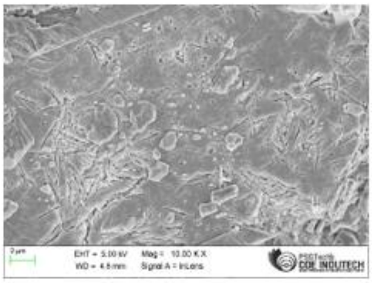
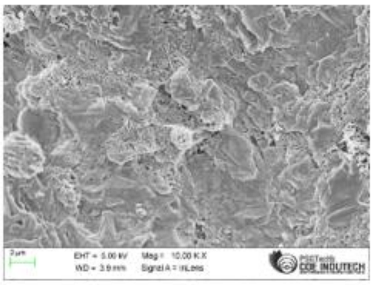
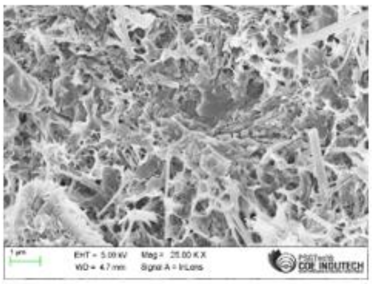
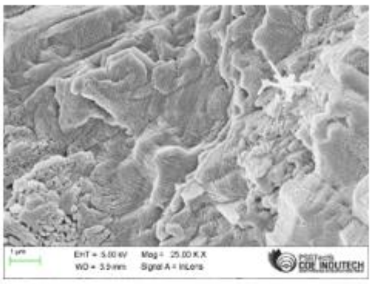
The above Table 4.6 displays the compressive strength results of bacteria-induced cement mortar cubes, showcasing an increase in strength compared to conventional cement cubes. This improvement is attributed to the addition of Actino bacteria, Bacillus alluvi and Pseudomonas aeruginosa contributing to enhanced strength.

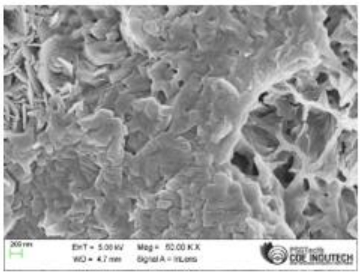
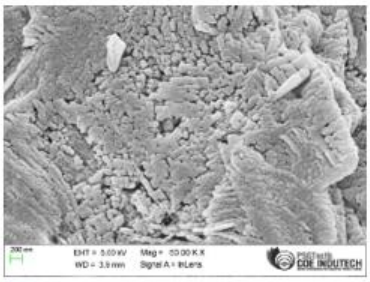
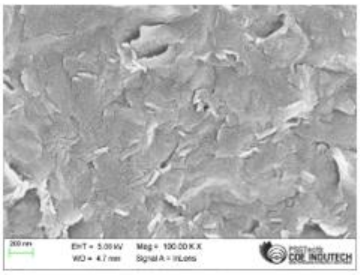
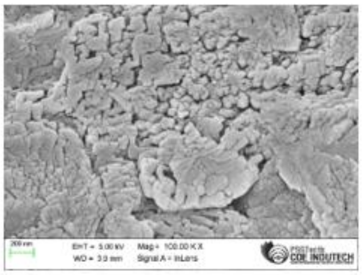
4.3.2 SCANNING ELECTRON MICROSCOPY ANALYSIS (SEM)

Scanning Electron Microscopy (SEM) offers a window into the microscopic realm. Through SEM analysis, we can scrutinize the surface morphology and particle size of the cement specimens. This detailed analysis helps us better understand how well the bioremediation process works. It helps us see the complex ways microorganisms, cement, and heavy metal pollutants interact with each other.

Table.4.7 SEM images of Maha cement – with and without bacteria induced cement mortar cubes

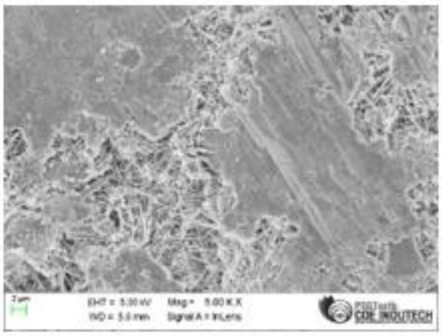
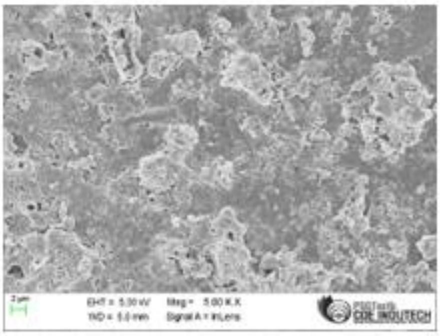
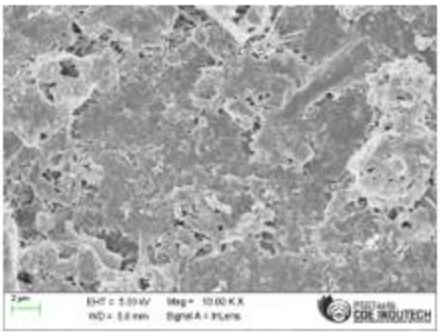
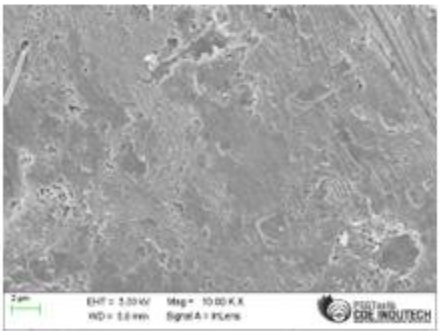
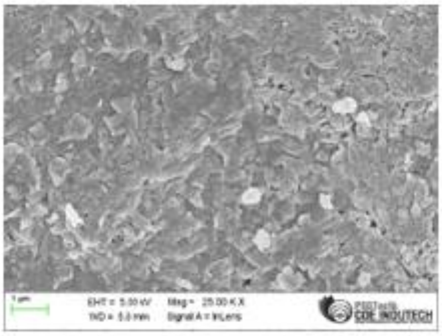
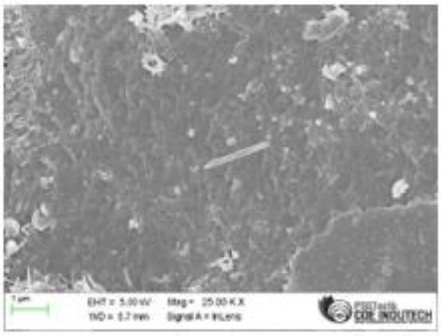
Maha cement (OPC) - with bacteria	Maha cement (OPC) - without bacteria
2µm and Magnification – 5.K X	
	

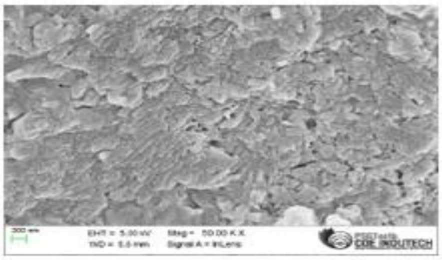
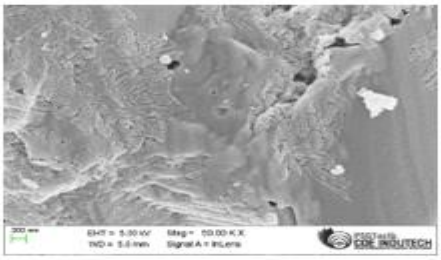
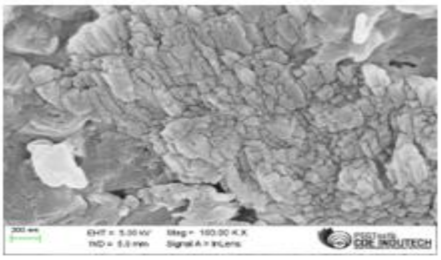
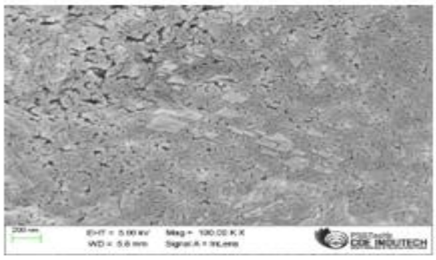
2µm and Magnification – 10.K X	
	
1µm and Magnification – 25.K X	
	

200 nm and Magnification – 50.K X	
	
200 nm and Magnification – 100.K X	
	

The above Table 4.7 displays the SEM images of the cement mortar cubes of Maha cement (OPC) that represents the presence of rod-shaped bacteria involved in calcite production, which contributes to an increase in the compressive strength of cement mortar cubes, where as in the conventional cement mortar cubes there is no calcite precipitation.

Table.4.7 SEM images of Ulltratech cement – with and without bacteria induced cement mortar cubes

Ultratech (OPC) cement - with bacteria	Ultratech(OPC)cement - without bacteria
2µm and Magnification – 5.K X	
	
2µm and Magnification – 10.K X	
	
1µm and Magnification – 25.K X	
	

200 nm and Magnification – 50.K X	
	
200 nm and Magnification – 100.K X	
	

The above Table 4.8 displays the SEM images of the cement mortar cubes of ultratech OPC cement that represents the presence of rod-shaped bacteria involved in calcite production, which contributes to an increase in the compressive strength of cement mortar cubes, where as in the conventional cement mortar cubes there is no calcite precipitation.

4.3.3 ENERGY DISPERSIVE X-RAY ANALYSIS (EDAX)

Energy Dispersive X-Ray Analysis (EDAX), is an X-ray technique used to identify the elemental composition of materials. It provides valuable insights into the chemical characterization of a sample by analyzing the X-rays emitted during interactions between X-ray excitation and the sample**.**

4.3.3.1 ELEMENTAL COMPOSITION OF MAHA CEMENT WITH BACTERIA

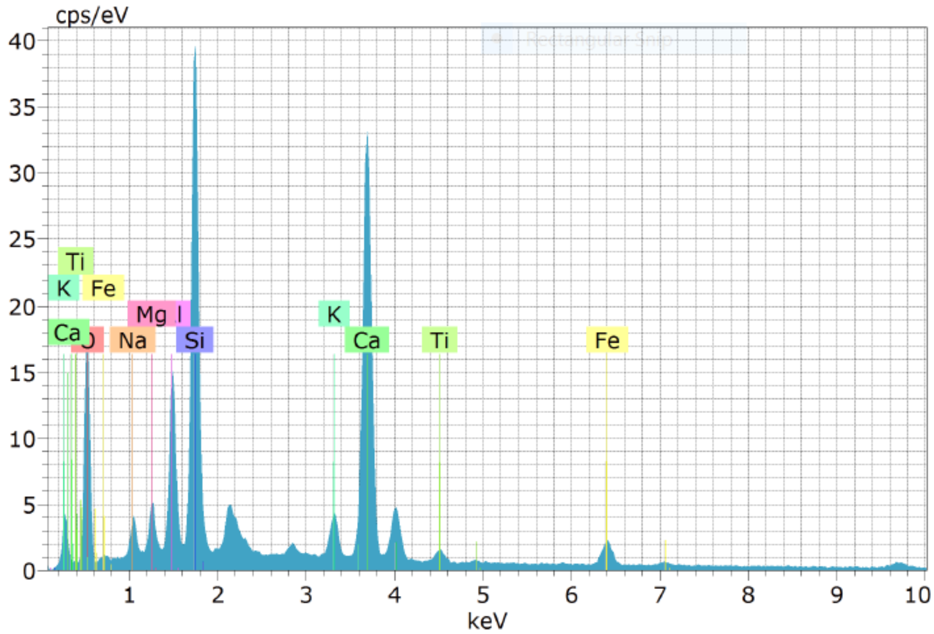


Fig.4.26 Elemental composition

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Oxygen	K-series	23.75	45.07	63.51	9.52
Calcium	K-series	13.56	25.73	14.47	1.28
Silicon	K-series	7.33	13.91	11.17	1.02
Aluminium	K-series	2.94	5.57	4.66	0.51
Iron	K-series	1.99	3.77	1.52	0.27
Sodium	K-series	0.98	1.87	1.83	0.29
Potassium	K-series	0.91	1.74	1.00	0.17
Magnesium	K-series	0.85	1.62	1.50	0.23
Titanium	K-series	0.38	0.72	0.34	0.13
	Total:	52.70	100.00	100.00	

Fig.4.27 Quantitative data of elemental composition

4.3.3.2 ELEMENTAL COMPOSITION OF MAHA CEMENT WITHOUT BACTERIA

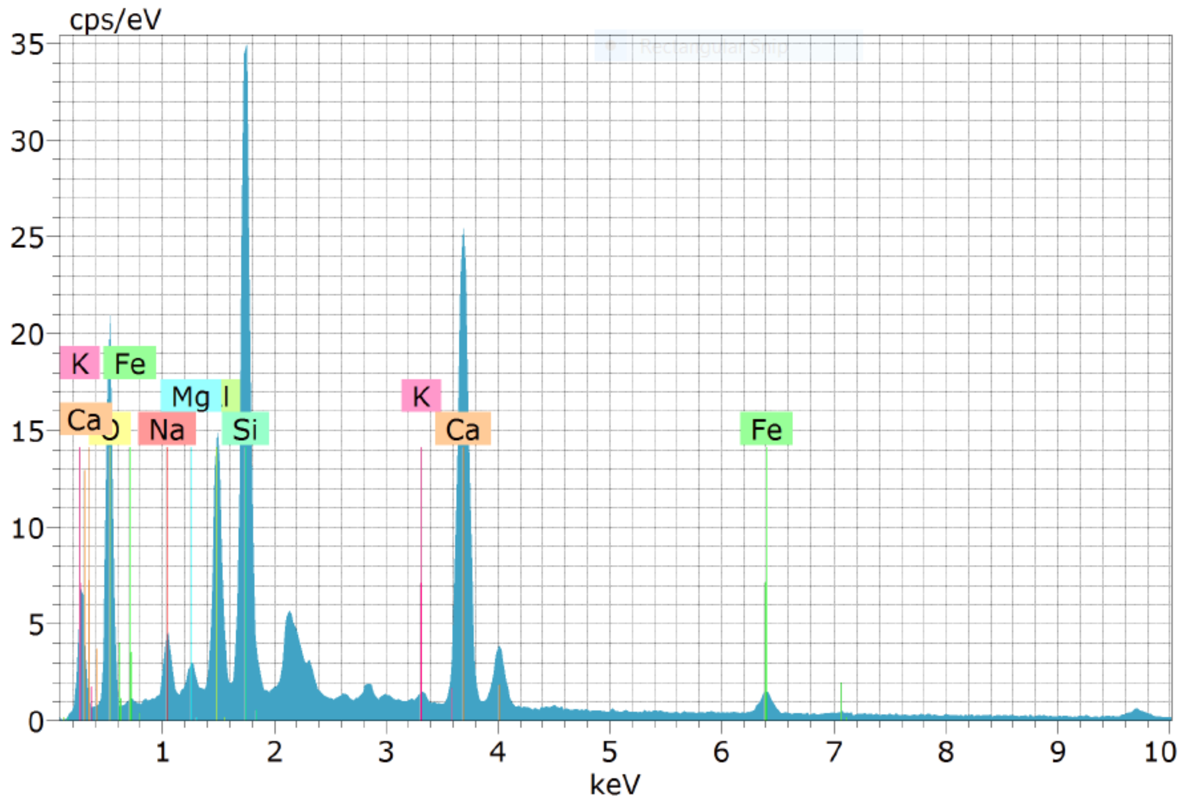


Fig.4.28 Elemental composition

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Oxygen	K-series	24.17	49.42	66.63	9.46
Calcium	K-series	11.21	22.92	12.34	1.07
Silicon	K-series	7.13	14.57	11.19	1.00
Aluminium	K-series	3.29	6.72	5.37	0.56
Sodium	K-series	1.40	2.87	2.70	0.37
Iron	K-series	1.27	2.60	1.00	0.21
Magnesium	K-series	0.40	0.82	0.73	0.16
Potassium	K-series	0.04	0.08	0.04	0.09
	Total:	48.90	100.00	100.00	

Fig.4.29 Quantitative data of elemental composition

4.3.3.3 ELEMENTAL COMPOSITION OF ULTRATECH CEMENT WITH BACTERIA

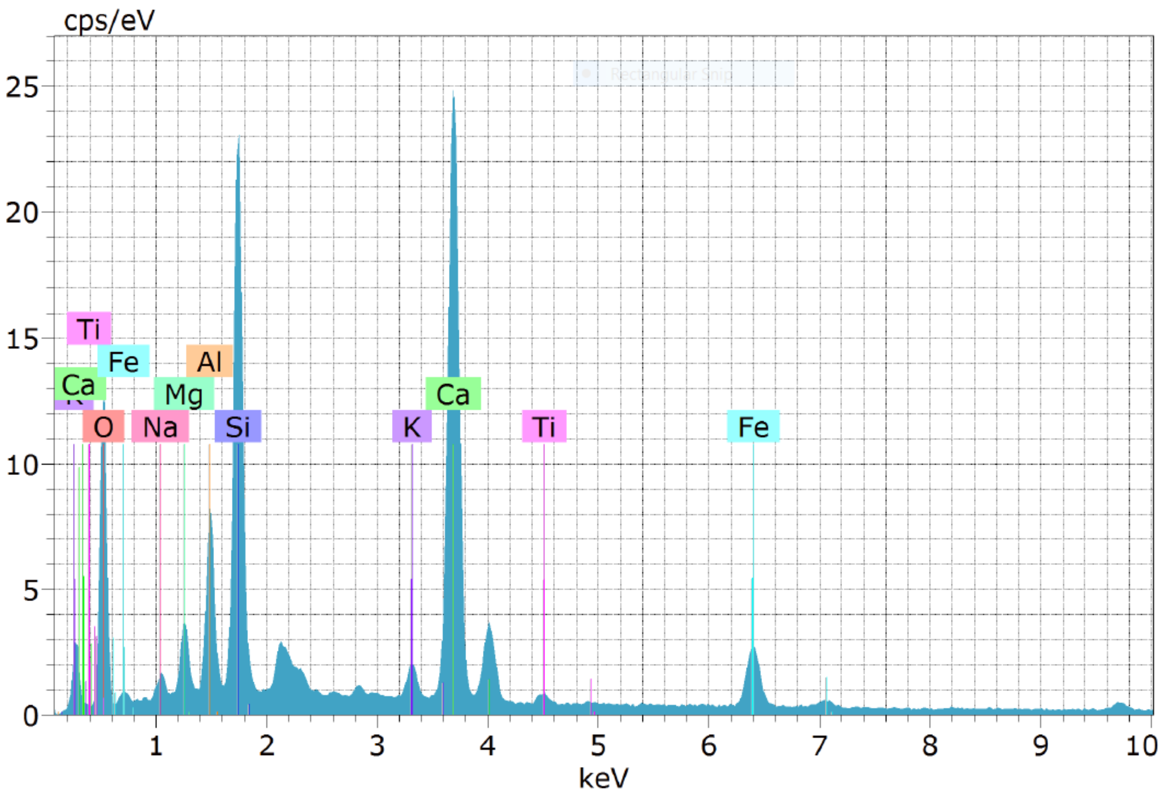


Fig.4.30 Elemental composition

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Oxygen	K-series	20.59	46.14	65.78	8.25
Sodium	K-series	0.24	0.54	0.54	0.14
Magnesium	K-series	0.76	1.71	1.60	0.22
Aluminium	K-series	1.83	4.09	3.46	0.35
Silicon	K-series	5.15	11.54	9.37	0.74
Potassium	K-series	0.24	0.54	0.32	0.11
Calcium	K-series	12.37	27.72	15.78	1.17
Iron	K-series	3.42	7.67	3.13	0.38
Titanium	K-series	0.03	0.06	0.03	0.08
	Total:	44.64	100.00	100.00	

Fig.4.31 Quantitative data of elemental composition

4.3.3.3 ELEMENTAL COMPOSITION OF ULTRATECH CEMENT WITH BACTERIA

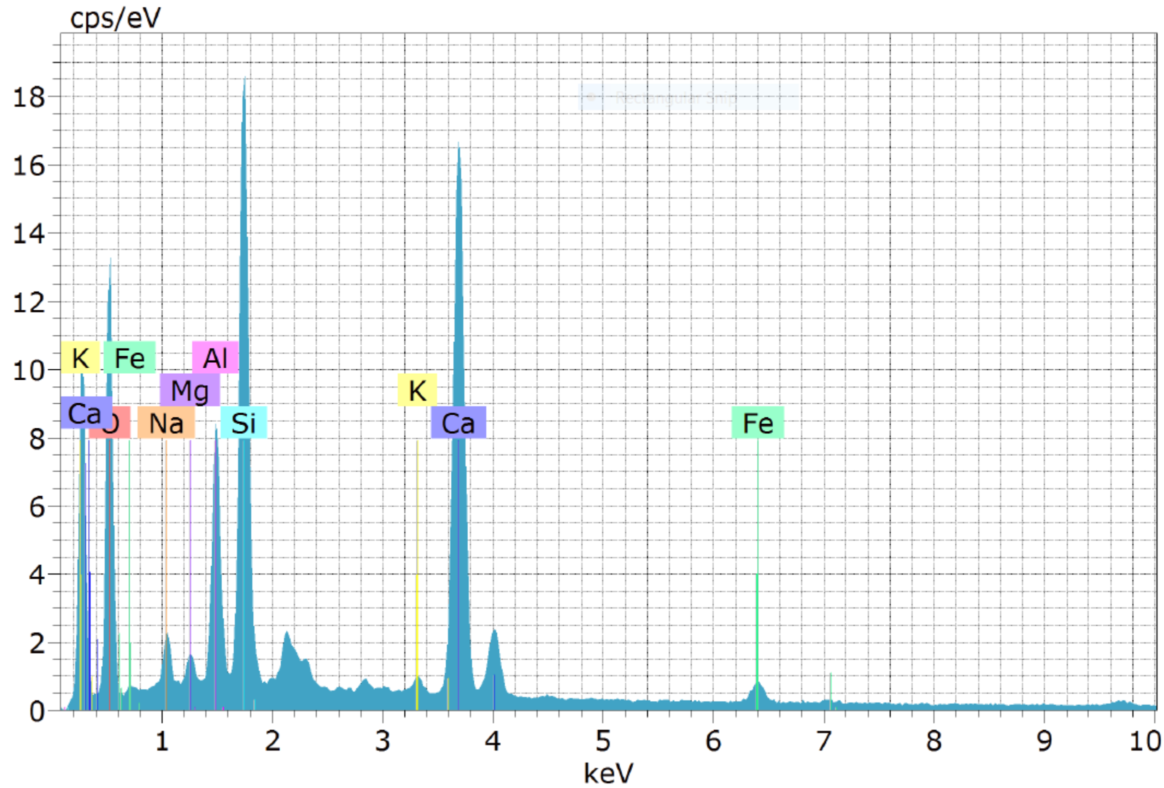


Fig.4.32 Elemental composition

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Oxygen	K-series	25.62	52.45	69.73	9.95
Sodium	K-series	1.07	2.20	2.03	0.31
Magnesium	K-series	0.29	0.59	0.52	0.14
Aluminium	K-series	2.89	5.92	4.66	0.50
Silicon	K-series	5.99	12.26	9.29	0.85
Calcium	K-series	11.81	24.17	12.83	1.12
Iron	K-series	1.11	2.27	0.87	0.20
Potassium	K-series	0.07	0.14	0.07	0.09
	Total:	48.84	100.00	100.00	

Fig.4.33 Quantitative data of elemental composition

ANALYSIS OF HEAVY METALS USING ATOMIC ABSORPTION SPECTROSCOPY

5.1 CONVENTIONAL CEMENT

Atomic Absorption Spectroscopy (AAS) analyses were carried out to further characterize the presence of heavy metals in cement.

5.1.1 ACID DIGESTION PROCESS OF CEMENT SAMPLES

The acid digestion process is vital for the chemical analysis of cement samples. Through finely grinding the solid cement and using a mixture of strong acids like hydrochloric acid and nitric acid, this process dissolves the cement matrix. The resulting solution, obtained after cooling and filtration, contains the dissolved components ready for further analysis. Here is the procedure for the acid digestion process:

- Weigh 1 gram of cement from each of the 8 samples and place them under a fume hood.
- Combine 12 ml of hydrochloric acid with 4 ml of nitric acid in a 3:1 ratio and add the mixture to the cement samples.
- Boil the samples to eliminate fumes, then let them cool for one hour.
- After cooling, introduce 50 ml of distilled water to each sample.
- And proceed to filter each sample using filter paper.

Fig.5.1 Cement samples of 1 gram

Fig.5.2 Hydrochloric and Nitric acid

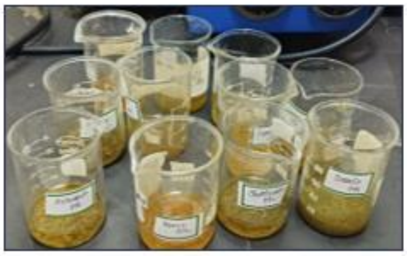


Fig.5.3 After the addition of acids

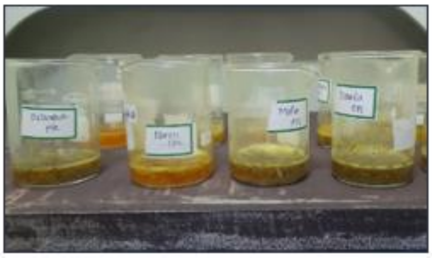


Fig.5.4 Samples after the removal of fume

Fig.5.5 Addition of 50ml of distilled water

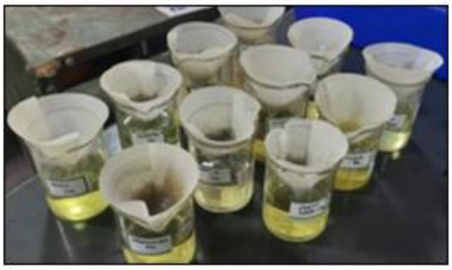


Fig.5.6 Filtration of samples

5.1.2. GRAPH

The Atomic Absorption Spectroscopy (AAS) results graph visually represents the concentration of elements in a sample. Peaks on the graph indicate absorption intensity, directly correlating with element concentration. The calibration curve establishes a relationship between absorbance and known concentrations, facilitating quantification of unknown samples. Reproducibility, noise reduction, and adherence to calibration standards ensure result accuracy. The graph's interpretation involves comparing unknown sample peaks to the calibration curve, with higher peaks signifying higher concentrations. Monitoring instrument performance and determining the limit of detection (LOD) contribute to quality control in AAS analysis.

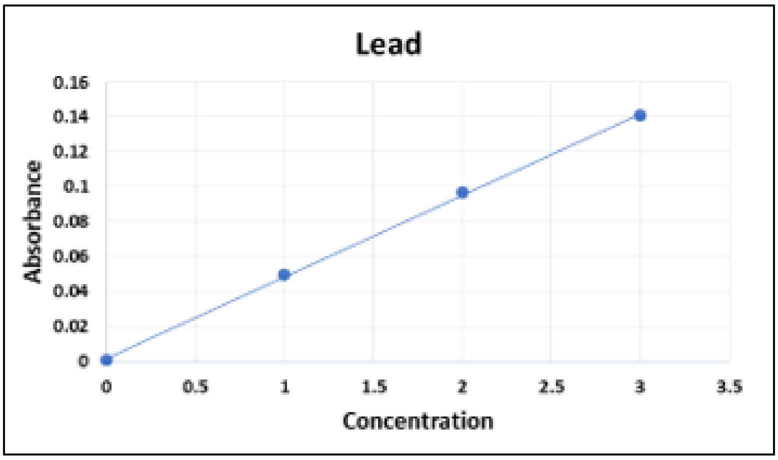


Fig.5.7 Absorbent Vs Concentration graph for Lead

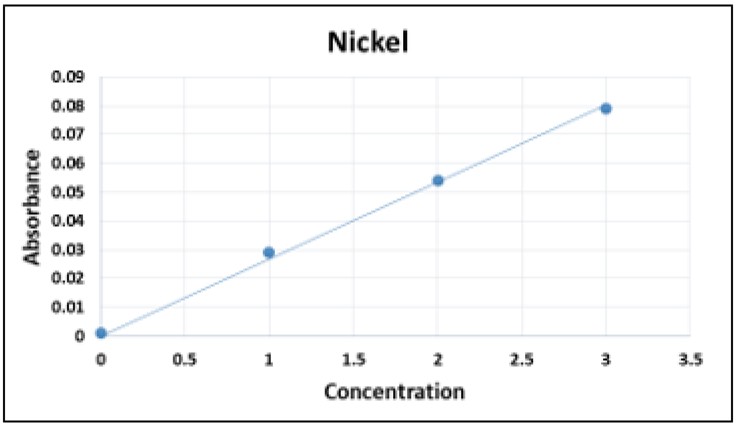


Fig.5.8 Absorbent Vs Concentration graph for Nickel

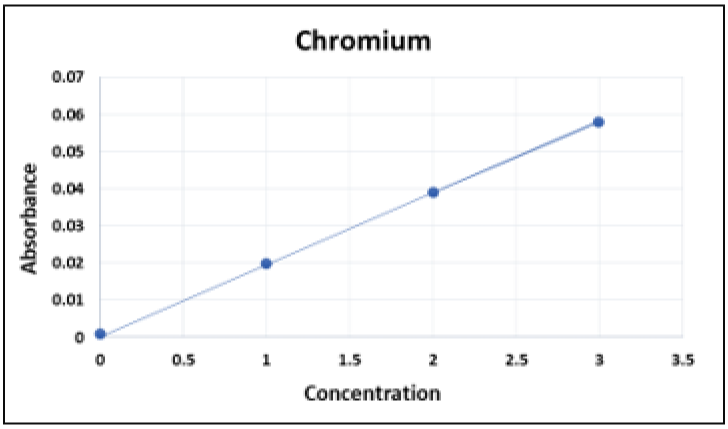


Fig.5.9 Absorbent Vs Concentration graph for Chromium

Table.5.1 Test results of concentration of heavy metals in conventional cement

S.no	Cement brand	Grade and type of cement	Heavy metals/Results (ppm)		
			Lead (Pb)	Nickel (Ni)	Chromium (Cr)
1	Brila shakti	PPC	34.5	34.08	66.73
2	Chettinadu	PPC	33.28	31.36	47
3	Coromandel	PPC	45.79	26.5	41.90
4	Dalmia	PPC	43	49.54	89.95
5	Maha	PPC	31.5	28	45.5
6	Ramco	PPC	50	55.19	87.5
7	Ultratech	PPC	30.44	37.79	39.49
8	Dalmia	53 & OPC	39	42	55.5

9	Maha	53 & OPC	44.18	36.45	69.5
10	Ramco	53 & OPC	50	64.5	50
11	Ultratech	53 & OPC	43	41.5	57

Above in Table 5.1, the outcomes of the AAS analysis reveal the concentrations of nickel, chromium, and lead in the eleven cement samples.

5.2 BACTERIA INDUCED CEMENT

Atomic Absorption Spectroscopy (AAS) analyses were carried out to further characterize the presence of heavy metals in cement.

Each bacteria of 1ml is added to every cement sample, with additional glucose provided as nutrient to sustain bacterial viability. The cement samples are then covered with Parafilm and incubated for four weeks to facilitate interaction. After this period, the samples undergo acid digestion before being analyzed using AAS.

5.2.1 ACID DIGESTION PROCESS

The acid digestion process is vital for the chemical analysis of cement samples. Through finely grinding the solid cement and using a mixture of strong acids like hydrochloric acid and nitric acid, this process dissolves the cement matrix. The resulting solution, obtained after cooling and filtration, contains the dissolved components ready for further analysis. Here is the procedure for the acid digestion process:

- Weigh 1 gram of cement from each of the 8 samples and place them under a fume hood.
- Combine 12 ml of hydrochloric acid with 4 ml of nitric acid in a 3:1 ratio and add the mixture to the cement samples.
- Boil the samples to eliminate fumes, then let them cool for one hour.
- After cooling, introduce 50 ml of distilled water to each sample.
- And proceed to filter each sample using filter paper.

Fig.5.10 Cement samples of 1 gram

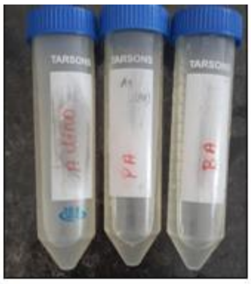


Fig.5.11 Bacteria

Fig.5.12 Addition of bacteria

Fig.5.13 Glucose is added to the samples as bacterial food

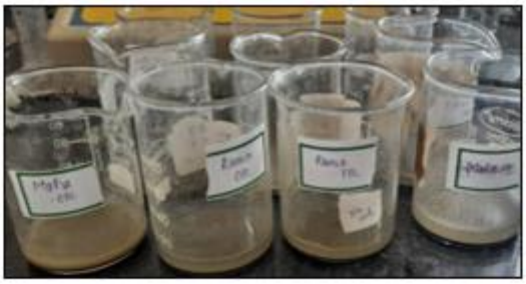


Fig.5.14 After the addition of bacteria

Fig.5.15 Samples covered with paraflim

5.4.2 GRAPH (Bacteria induced cement samples)

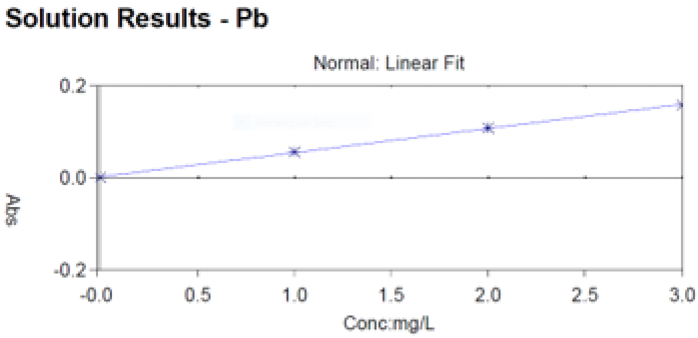


Fig.5.16 Absorbent Vs Concentration graph for Lead

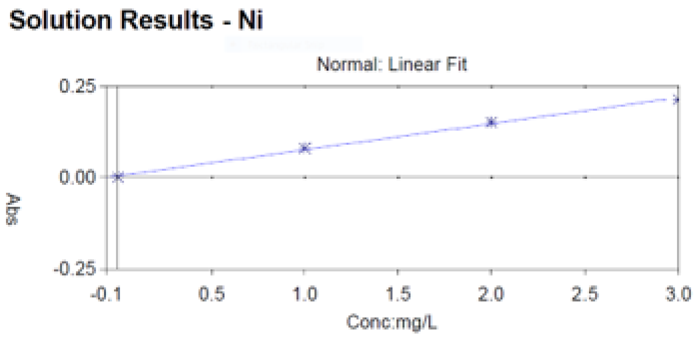


Fig.5.17 Absorbent Vs Concentration graph for Nickel

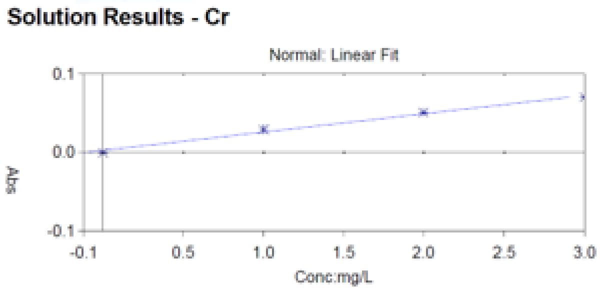


Fig.5.18 Absorbent Vs Concentration graph for Chromium

Table.5.2 Test results showing the concentration of heavy metals in bacteria induced cement samples

S.no	Cement brand	Grade and type of cement	Heavy metals/Results (ppm)		
			Lead (Pb)	Nickel (Ni)	Chromium (Cr)
1	Brila shakti	PPC	29	29	49.5
2	Chettinadu	PPC	30.31	29.5	38.03
3	Coromandel	PPC	33.26	24.48	39.5
4	Dalmia	PPC	34.5	48	82
5	Maha	PPC	28.87	27.55	36.76
6	Ramco	PPC	17.40	43	67.37
7	Ultratech	PPC	28.5	28	31.49
8	Dalmia	53 & OPC	38.28	41.18	54.48
9	Maha	53 & OPC	42	35.5	57.52
10	Ramco	53 & OPC	16.10	40.70	37.36
11	Ultratech	53 & OPC	29.14	40.62	10.67

The table 5.2 above shows the heavy metal concentration results in bacteria-induced cement samples. It indicates that the addition of bacteria leads to a more significant reduction in heavy metal concentrations compared to the previous results.

RESULTS AND DISCUSSION

6.1 INTIAL AND FINAL SETTING TIME

The initial and final setting time of all the collected cement brands are within the limits as per IS 269:2015 and IS 1489: Part 1:2015.

6.2 STANDARD CONSISTENCY TEST

The standard consistency tests for all collected cement brands confirm that the water content for Grade 53

OPC cement falls between 25-35%, while for PPC cement, it's between 32-36%, as per IS 269:2015 and IS 1489: Part 1:2015.

6.3 DRY SEIVING METHOD

The fineness for all the collected cement brands of grade 53 & OPC and PPC is less than 10 % as per IS 4031 part 1: 1996.

6.4 SPECIFIC GRAVITY OF CEMENT

The specific gravity for all the collected cement brands of grade 53 & OPC and PPC is between 3.1 and 3.15 as per IS 4031 part 11: 1998.

6.5 ATOMIC ABSORPTION SPECTROSCOPY

- The table 6.1 illustrates that comparison of test results indicates the concentration of heavy metals in both conventional cement and bacteria induced cement.
- It indicates that the addition of bacteria leads to a more significant reduction in heavy metal concentrations.
- The results of the Atomic Absorption Spectroscopy (AAS) analysis indicate that the concentrations of heavy metals are generally within the permissible limits
  as per World health organization (WHO) shown in table 6.2

Table.6.1 Comparison of test results -Atomic absorption spectroscopy

S.no	Cement brand	Grade and type of cement	Heavy metals Concentration (ppm) [Conventional cement]			Heavy metals Concentration (ppm) [bacteria-induced cement]			% Reduction		
			Pb	Ni	Cr	Pb	Ni	Cr	Pb	Ni	Cr
1	Brila shakti	PPC	34.5	34	66.7	29	29	49.5	15.94	14.9	25.82
2	Chettinadu	PPC	33.28	31	47	30.31	29.5	38.03	8.92	5.93	19.09
3	Coromandel	PPC	45.79	27	41.9	33.26	24.48	39.5	27.36	7.62	5.73
4	Dalmia	PPC	43	50	90	34.5	48	82	19.77	3.11	8.84
5	Maha	PPC	31.5	28	45.5	28.87	27.55	36.76	8.35	1.61	19.21
6	Ramco	PPC	50	55	87.5	17.4	43	67.37	65.2	22.1	23.01
7	Ultratech	PPC	30.44	38	39.5	28.5	28	31.49	6.37	25.9	20.26
8	Dalmia	53 & OPC	39	42	55.5	38.28	41.18	54.48	1.85	1.95	1.84
9	Maha	53 & OPC	44.18	36	69.5	42	35.5	57.52	4.93	2.61	17.24
10	Ramco	53 & OPC	50	65	50	16.1	40.7	37.36	67.8	36.9	25.28
11	Ultratech	53 & OPC	43	42	57	29.14	40.62	10.67	32.23	2.12	81.28

Table.6.2 Maximum allowable limit of the heavy metals in cement as per WHO

S.no	Heavy metal	Maximum allowable limit
1	Lead (Pb)	50
2	Nickel (Ni)	200
3	Chromium (Cr)	100

6.6 COMPRESSIVE STRENGTH

Table.6.3 Comparison of test results - Compressive strength test

S.no	Cement brand	Grade and type	Compressive strength								% Increase	
			Conventional cement mortar cubes				Bcatria induced cement mortar cubes					
			7th Day (N/mm²)		28th day(N/mm²)		7th Day (N/mm²)		28th day(N/mm²)			
			Individual cubes	Avg	Individual cubes	Avg	Individual cubes	Avg	Individual cubes	Avg	7th Day (N/mm²)	28th day (N/mm²)
1	Brila shakti	PPC	26.4	28.8	45.6	45.6	34.6	33.8	49.8	50.4	17.36	10.53
			29.8		45.2		33.6		50.6			
			30.4		46.2		33.4		50.8			
2	Chettinadu	PPC	28	27.2	45.4	46	30.8	30.2	50.2	50.2	11.03	9.13
			27.8		46.4		30.2		49.8			
			25.8		46.2		29.2		50.4			
3	Coromandel	PPC	27.2	26.2	44.6	42.6	30.6	30.8	46.4	47.2	17.56	10.8
			25		42.6		31.4		47.8			
			26.4		40.8		30.4		47.4			
4	Dalmia	PPC	26.1	24.8	40.6	43.7	27.4	28.3	47.8	46.9	14.11	7.32
			24.3		43.2		28.8		46.8			
			24.1		47.4		28.8		46.2			
5	Maha	PPC	24.08	25.96	47.4	46.6	30.8	30.1	49.8	50.13	15.79	7.58
			27		47.2		30.2		50.8			
			26.8		45.4		29.2		49.8			
6	Ramco	PPC	27	27.4	48.4	48.2	29.8	29.9	50.8	50.3	8.98	4.36
			27.4		47.6		29.6		49.8			
			28		48.6		30.2		50.4			
7	Ultratech	53 & OPC	27	26.36	40.8	42.7	31.8	31.1	45.4	45.8	17.83	7.26
			25		44.6		30.8		45.8			
			27.08		42.8		30.6		46.2			
8	Dalmia	53 & OPC	27.4	25.1	45.8	45.8	31.8	29	50.8	50.4	15.54	10.04
			24.5		45.2		30.4		50.6			
			23.4		46.4		30.8		49.8			
9	Maha	53 & OPC	26.08	27.82	47.4	46.6	32.4	32.2	50.4	49.9	15.74	7.08
			29		47.2		31.8		49.8			
			28.4		45.4		32.6		49.6			
10	Ramco	53 & OPC	24.1	23	47.2	48.8	26.8	26.4	51.4	51.8	14.78	6.15
			23		49		26.6		52.4			
			22.1		50.4		25.8		51.6			
11	Ultratech	53 & OPC	26	25.8	42.8	43.6	30.2	29.8	52.8	52.4	15.5	20.18
			26.4		42.4		29.8		51.8			
			25.2		45.6		29.4		52.4			

- The table 6.3 above shows a comparison of compressive strength test results between conventional cement mortar cubes and bacteria-induced cement mortar cubes.
- It demonstrates that the bacteria-induced cement mortar cubes exhibit greater strength compared to conventional cement, indicating that the addition of bacteria has enhanced the mechanical properties of the cement mortar cubes.

6.7 SCANNING ELECTRON MICROSCOPY ANALYSIS (SEM)

- The observed rod-shaped bacteria in the SEM images corroborate previous studies indicating their role in bio mineralization processes within cementitious materials.
- The formation of calcite by these bacteria within the cement mortar matrix demonstrates their potential to enhance the mechanical properties of construction materials, particularly in terms of compressive strength.

6.8 ENERGY DISPERSIVE X-RAY ANALYSIS (EDAX)

- The EDAX analysis provides valuable insights into the elemental composition of the cement mortar cubes, revealing a notable increase of 20.9% and 4.7% in calcium content in the bacterial-induced samples compared to their conventional counterparts.
- The higher calcium content in the bacterial-induced cement mortar cubes underscores the active role played by bacteria in facilitating calcium precipitation, a crucial process for enhancing the mechanical properties of the material.

CONCLUSION

- Bioremediation employing microorganisms to reduce heavy metal content in cement shows significant promise.
- The physical properties of cement were evaluated as per IS codal provisions.
- Table 6.1 highlights a substantial decrease in heavy metal concentrations in bacteria-treated cement compared to conventional cement.
- Atomic absorption analysis in Table 6.2 indicates heavy metal concentrations generally within WHO limits. Addition of bacteria proves effective in reducing heavy metal concentrations, demonstrating bioremediation's potential to address environmental concerns related to heavy metal leaching in cement.
- Enhanced mechanical properties in bacteria-treated cement mortar cubes, as shown in Table 6.3, suggest potential for more durable and sustainable construction materials, aligning with industry sustainability goals and prompting further research into microbial interventions for broader applications. This study lays groundwork for future investigations, highlighting bioremediation's dual impact on environmental and structural aspects of cement materials.
- SEM images confirm the presence of rod-shaped bacteria, indicating their role in bio mineralization within cementitious materials. Calcite formation by these bacteria enhances mechanical properties, especially compressive strength.
- Additionally, EDAX analysis reveals a notable increase of 20.9% and 4.7% in calcium content in bacterial-treated samples, underscoring the bacteria's crucial role in strengthening materials through calcium precipitation. This evidence highlights the potential of bacterial interventions in enhancing the mechanical integrity of cementitious materials for more sustainable construction practices.

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