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Synergistic Antibacterial Effects of Saponified Waste Oil and Cetrimonium Chloride (Ctac) in Cement Pastes

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Abstract

Microbial colonization activity in cement pastes can lead to biodeterioration, compromising the structural integrity over time. This study investigates the antibacterial performance of cement pastes modified with saponified cooking oil (SCO) and cetrimonium chloride (CTAC). Different doses of CTAC and SCO were blended with cement paste on four bacterial strains: Escherichia coli and Pseudomonas aeruginosa (Gram-negative), Bacillus subtilis, and Staphylococcus aureus (Gram-positive). The results showed that B. subtilis revealed the highest sensitivity to CTAC's antibacterial activity, especially at high doses (up to 1%). This effect was enhanced by adding SCO, indicating a clear synergistic interaction. Gram-positive bacteria generally reacted more strongly to the modified cement paste comparing with Gram-negative bacteria. The results demonstrated the potential of CTAC/SCO-modified cement for use in microbial-sensitive environments such as hospitals, and food processing facilities.

Keywords: *Cementitious materials, Cetrimonium chloride (CTAC), Saponified cooking oil (SCO), Antibacterial performance, Gram-positive bacteria, Gram-negative bacteria, Zone of inhibition.*

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I. INTRODUCTION

Cement is the most widely used material in the construction sector; however, the durability of cement structures faces some challenges such as exposure to environmental conditions [1], material quality [2], mechanical stress [3], poor construction practices [4] and biological deterioration [5].

The sensitivity of cement structures to biological deterioration, arising from the growth of microorganisms like bacteria, has been assigned to be a significant concern for engineering applications and environments [6,7]. This deterioration is reported to be a major cause of reduced durability and loss of aesthetic value, as well as damage to heritage building structures [8], drainage systems [9] and offshore platforms [5].

Several efforts modified cement chemical composition with additives (sustainable and environmentally friendly) to make cement structures resistant to biological degradation such as antimicrobial concrete (AMC) [10, 11]. Researchers have shown increasing interest in AMCs in various settings such as hospitals, laboratories, and food processing plants; however, there is growing concern about the reactivity, cost, and health risks of these synthetic antimicrobial agents.

One promising antibacterial application in concrete and cement pastes involves cationic surfactants, specifically quaternary ammonium compounds such as cetrimonium chloride (CTAC). CTAC are attracted to the negatively charged surfaces of bacterial cell membranes, disrupting their structure and function, which lead to leakage of cellular contents and cell death. CTAC is more effective at alkaline media such as, cement paste media, where the negative charge of bacterial interfaces is more pronounced at these pH levels, enhancing their binding and interaction [12]. However, practical implementation remains limited due to cost considerations and the absence of clear regulatory guidelines [12].

To reduce costs and utilize environmentally friendly materials in antimicrobial concrete (AMC), saponified cooking oil (SCO) can enhance antimicrobial effectiveness. Several studies have indicated that the fatty acids in SCO act as surfactants by raising the alkalinity of the mixture and by disrupting microbial cell membranes by interacting with lipid

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bilayers [12, 13]. Moreover, saponified cooking oil (SCO) can reduce bacterial adhesion and biofilm formation on concrete surfaces by altering surface hydrophobicity [14].

According to [15] study, there is a knowledge gap regarding the use of mixtures of ammonium compounds and organic oils as admixtures that effectively inhibit microbial growth and concrete degradation, thus achieving sustainable development. Thus, this study aims to verify the effectiveness of cationic surfactant and saponified cooking oil on the ability of cement pastes to resist Gram-negative and Gram-positive bacteria.

II. Materials

1. Ordinary Portland Cement (OPC) (CEM I 52.5N): was obtained from El Arish Cement Factory. Table 1 contains its chemical composition as supplied by the factory.
2. Used cooking sunflower oil: its key physicochemical characteristics are listed in Table 2.
3. Potassium hydroxide (KOH): was purchased from Merck and used to prepare 0.1 M potassium hydroxide (KOH) solution.
4. Cetrimonium Chloride (CTC): a commercial-grade surfactant used as an admixture purchased from Lerochem and used as received as given in Table 3.
5. Dimethyl Sulfoxide (DMSO): from Sigma-Aldrich and used as received.

III. Methods

A. Oil Collection and Filtration:

The used sunflower cooking oil was collected from the researcher's home in Gaza, Palestine. The impurities were purified using three steps: the first was gravity precipitation to separate large impurities, then filtration using a 150-micrometer sieve, and finally vacuum filtration to remove impurities less than 150 micrometers. All the treated samples of used cooking oil will be stored in plastic drums.

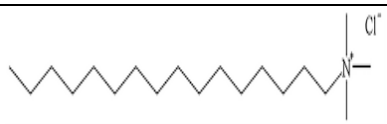
Table 1. The chemical composition of the used ordinary Portland cement (OPC).

Oxide	(%)	Oxide	(%)
SiO_2	22.12	Na_2O	0.26
Al_2O_3	5.56	K_2O	0.11
Fe_2O_3	3.69	Cl-	0.02
CaO	62.87	MgO	2.36
SO_3	0.91	Free C_aO	0.92

Table 2. Physicochemical properties of Sunflower oil (before and after cooking)

Property	Before Cooking	After Cooking
Density (g/cm³) at 20°C	0.912	0.926
Refractive Index at 20°C	1.461	1.474
Saponification Value (mg KOH/g oil)	191	189.5
Iodine Value (g I_2/100 g oil)	136	133.5
Acid Value (mg KOH/g oil)	2.10	3.70
Peroxide Value (meq O_2/kg oil)	0.77	6.80

Table 3. Specification of Cetrimonium Chloride solution

Parameter	Attribute
Chemical Name	Cetyltrimethylammonium Chloride (CTAC)
Chemical Formula	$C_{19}H_{42}ClN$
INCI Name	Cetrimonium Chloride
Molar Mass	284.5 g/mol
Density	0.95 g/cm³ (at 20 °C)
Solubility	Miscible in water
Physical State	Typically available as a 30% aqueous solution
Grade Used	Commercial Grade
pH at 25C^0	6-8
Character	cationic surfactant
Appearance	Clear colorless liquid
Structure	

B. Saponification of Used Cooking Oil:

The used oil undergoes partial cold saponification with 0.1 M KOH under continuous stirring (200 rpm) for 30 min at pH 8 and 25°C. About 20% of the oil remained untreated. The Saponified Cooking Oil (SCO**)** was later used to prepare cement paste samples.

C. Cement Paste Preparation

Ten cement paste mixes were prepared using 1500 g of cement and 650 g of water (W/C ratio of 0.43) [16]. The formulations included: a reference mix without additives; three mixes containing saponified cooking oil (SCO) at 1%, 2.5%, and 5% (by cement weight); three mixes with cetrimonium chloride (CTAC) at 0.1%, 0.5%, and 1%; and three combination mixes prepared by dissolving 1% CTAC with SCO at 1%, 2.5%, and 5% before adding to the cement as shown in Table 4 . We selected 1% CTAC for combination with the three SCO samples, as it demonstrated the optimum antimicrobial performance compared to the lower concentrations of 0.1% and 0.5% [17].

Each mixture was stirred vigorously for about three minutes, then poured into 12 cm × 4 cm × 4 cm molds. Samples were kept at approximately 100% relative humidity at room temperature for 24 hours, followed by curing under water for up to 28 days. After curing, the hardened pastes were ground and suspended in sterile distilled water for subsequent testing.

TABLE 4. NUMBER OF CEMENT PAST SAMPLES WITH/OUT SCO AND CTAC

# of sample	Cement paste	% SCO	% CTAC
1 (no additive)	*	-	-
2	*	1	-
3	*	2.5	-
4	*	5	-
5	*	-	0.1
6	*	-	0.5
7	*	-	1
8	*	1	1
9	*	2.5	1
10	*	5	1

D. Solution Preparation and Antibacterial Activity Testing:

The ten ground paste samples were dissolved in dimethyl sulfoxide (DMSO) to prepare concentrated stock solutions. Then, these solutions were diluted with a complete growth medium to reach the following final concentrations before treatment: 12.5, 25, 50, 100, 200, and 400 µM. For all samples the concentration of DMSO in the growth medium did not exceed 0.3% (v/v) to avoid any toxic effects of the solvent itself on the bacterial cells.

E. Bacterial Culture Preparation

Clinical strains of bacterial strains were isolated from hospital wastewater. All bacterial strains were cultured in Brain Heart Infusion Broth (BHIB) at 37°C for 24 hours under static conditions before antibacterial testing. Bacterial identification was confirmed by standard biochemical assays and Gram staining following CLSI guidelines. The turbidity of the cultures was adjusted to match the 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). For broth-based testing, 100 µL of each bacterial suspension was transferred into 96-well microtiter plates containing test samples.

F. Microbiological Procedure:

The antibacterial performance of the modified cementitious materials was assessed using both the microbroth dilution method and the agar well diffusion assay, targeting two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). These strains were selected to evaluate the spectrum of antibacterial activity across differing cell wall structures.

1. Agar Well Diffusion *Assay*

To complement the broth assay, a zone of inhibition (ZoI) test was conducted. Mueller-Hinton agar plates were inoculated by spreading each bacterial suspension evenly over the surface. Wells of 6 mm diameter were punched into the agar, and 100 µL of the cement extract was added to each well. Plates were left at room temperature for 1 hour to allow for diffusion and then incubated at 37°C for 18–24 hours. Zones of inhibition were measured using a digital caliper to assess antibacterial efficacy [18].

2. Microbroth Dilution (MBD) with Tetrazolium Chloride *(TTC)*

The antibacterial activity in liquid media was assessed using the tetrazolium chloride (TTC) viability indicator. Cement suspensions were introduced into bacterial cultures in microtiter plates. After incubation at 37°C for 24 hours, 10 µL of 0.05% TTC solution was added to each well. The presence of a red color indicated metabolic activity and thus bacterial survival, while the absence or reduction of color signified inhibition [19].

This dual-method approach provided both qualitative (ZoI) and semi-quantitative (TTC colorimetric) insights into the antimicrobial performance of the various cement formulations. All measurements were performed in triplicate for each strain.

G. Statistical Methods

The mean and standard deviations (SD) of zone of inhibition for SCO, CTAC and three mixes were determined. To test the hypothesis that there is no significant difference in average zone of inhibition in SCO, CTAC and three CTAC and SCO mixes, one way ANOVA test were used at p-value of 0.05.

IV. RESULT AND DISCUSSION:

A total of 11 mixes , 10 mixes from Table 4 and DMSO sample, were tested for antibacterial activity against four clinical bacterial species Staphylococcus aureus and Bacillus subtilis (Gram-positive) and Escherichia coli and Pseudomonas aeruginosa (Gram-negative) bacteria using the zone of inhibition (ZoI) method.

A. Performance of Control Samples (DMSO and Blank)

The negative control (DMSO and blank cement paste) showed no inhibition zone against any of the tested bacterial strains (S. aureus, B. subtilis, E. coli, and P. aeruginosa), which evidence that the cement matrix and the DMSO have no antimicrobial properties, thus validating the subsequent results observed in the additive-containing formulations as shown in Table 5.

TABLE 5. ZONE OF INHIBITION (MM) MEAN ±SD FOR DIFFERENT CEMENT PASTE FORMULATIONS AGAINST TESTED BACTERIA

Sample	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*E. coli*
DMSO	–	–	–	–
Blank (no additive)	–	–	–	–
1% SCO	7.27±0.06	8.19±0.04	6.60±0.10	8.19±0.05
2.5% SCO	7.65±0.03	9.08±0.11	6.86±0.03	8.19±0.14
5% SCO	7.98±0.08	9.47±0.13	7.11±0.07	8.87±0.05
0.1% CTAC	9.65±0.11	11.10±0.06	8.66±0.07	9.85±0.03
0.5% CTAC	11.34±0.06	13.65±0.05	10.61±0.09	12.20±0.08
1% CTAC	13.09±0.13	16.03±0.09	11.13±0.05	13.70±0.06
Mix1 (1% CTAC + 1 SCO)	15.56±0.04	17.22±0.10	12.09±0.05	14.40±0.05
Mix2 (1% CTAC + 2.5% SCO)	16.16±0.10	17.72±0.15	12.35±0.08	14.35±0.12
Mix 3(1% CTAC + 5% SCO)	17.48±0.02	18.59±0.13	12.39±0.05	14.71±0.03

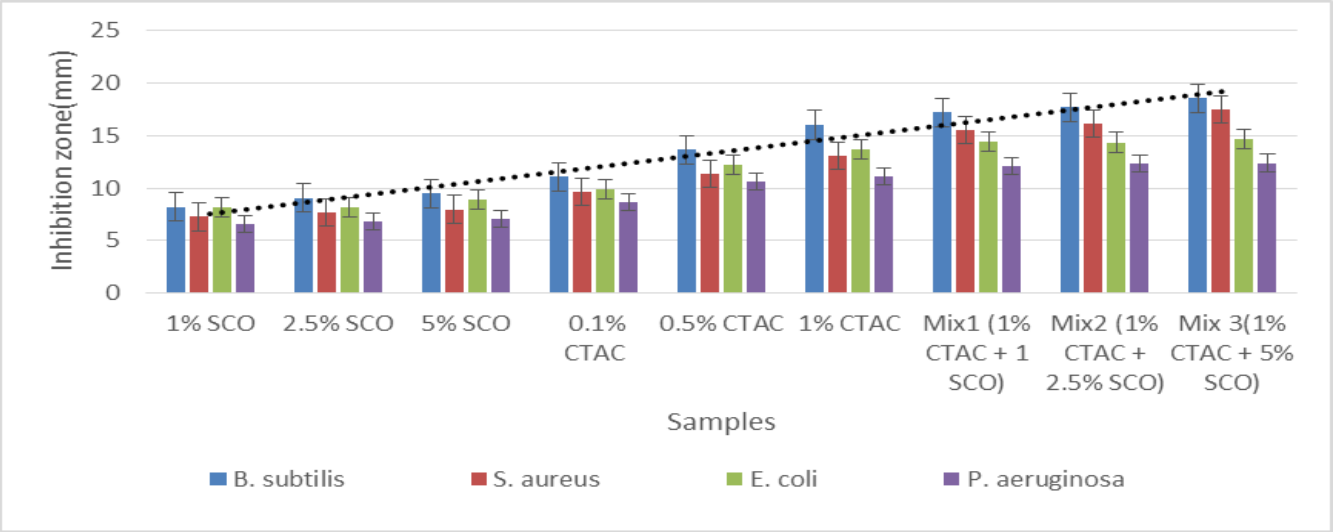


Fig. 1: Inhibition zone diameters of cement paste formulations against different bacterial strains

B. Antibacterial Effect of SCO

SCO is the potassium salt of fatty acids (soap) and belongs to the anionic surfactants.The results from Table 5 and Figure 1 indicated a moderate antibacterial activity for Gram-positive bacteria at 5% SCO mix. The inhibition zone reached 7.98±0.08mm for *S. aureus* and 9.47±0.13 mm for *B. subtilis*. For Gram-negative species, the inhibition zone reached 7.11±0.07mm for *P. aeruginosa* and 8.87±0.05mm for *E. coli*.

The inhibition zone increased steadily as the concentration increased from 1% to 5%, suggesting a possible dose-dependent response for the four species**.** As shown in Figure 1, inhibition zones for *B. subtilis* increased consistently from 8.19±0.04 mm at 1% to 9.47±0.13 mm at 5%. A one-way ANOVA showed a statistically significant difference in inhibition zones for *B. subtilis* among the three SCO concentrations**,** F(39.59), p = 0.001. Post hoc analysis using Tukey's HSD indicated that 5% SCO mix scored significantly higher than 1% SCO mix and 2.5% SCO mix.

Although increasing the SCO doses in the mixes improved antibacterial efficiency, the increase was not linear and did not correspond proportionally to the doubling of the SCO concentration. The observed improvements were relatively modest. This may be attributed to the accumulation of saponified oil within the cement matrix at higher concentrations, which likely increases viscosity and restricts the diffusion of active compounds to the bacterial surface.

SCO as an anionic surfactant has negatively charged groups present at its carboxylate hydrophilic head when it is in solution. This negative charge is very effective in solubilizing proteins, as it breaks non-covalent bonds within and between proteins, thus denaturing them [20]. These surfactants have a moderate antibacterial activity, which can be enhanced in the presence of low concentrations of divalent cations, and their efficacy becomes comparable to cationic surfactants [21].

The results confirmed the conclusions of previous studies that SCO, as an anionic activator, can act as a supporting factor in enhancing the antimicrobial action when used with other active compounds [22,23,24].

C. Antibacterial Effect of CTAC

As the CTAC concentration increased from 0.1% to 1%, there was a progressive increase in the inhibition zone diameters across all tested strains as shown in Table 5 and Figure 1. At 1% CTAC, the zones reached 13.09±0.13mm for S. aureus, 16.03±0.09mm for B. subtilis, 13.70±0.06mm for E. coli, and 11.13±0.05mm for P. aeruginosa .

D. Antibacterial Effect of CTAC

As the CTAC concentration increased from 0.1% to 1%, there was a progressive increase in the inhibition zone diameters across all tested strains as shown in Table 5 and Figure 1. At 1% CTAC, the zones reached 13.09±0.13mm for S. aureus, 16.03±0.09mm for B. subtilis, 13.70±0.06mm for E. coli, and 11.13±0.05mm for P. aeruginosa .

The inhibition zone increased steadily as the concentration increased from 0.1% to 1%, suggesting a possible dose-dependent response for the four species**.** As shown in Figure 1, inhibition zones for *B. subtilis* increased consistently from 11.10±0.06mm at 0.1% to 16.03±0.09mm at 1%. A one-way ANOVA showed a statistically significant difference in inhibition zones for *B. subtilis* among the three CTAC concentrations, F(1120), p = 0.001. Post hoc analysis using Tukey's HSD indicated that 1% CTAC mix scored significantly higher than 0.1% CTAC mix and 0.5% CTAC mix .This trend suggests that higher concentrations of CTAC may enhance antimicrobial activity.

CTAC is a cationic surfactant and a quaternary compound with an alkyl chain length typically of C19, resulting in high water solubility. CTAC particularly when applied at alkaline pH like cement pastes, also shows a high affinity for the interfaces of all two microbial classes of interest due to the negative charge of the microbial interfaces. According to [25]

study, the mechanism of CTAC involves adsorption and penetration of the bacterial cell wall, followed by interaction with the cytoplasmic membrane, leading to membrane disruption, leakage of low molecular weight intracellular materials, degradation of proteins and nucleic acids, and ultimately resulting in cell lysis and death.

E. Synergistic Effect of CTAC and SCO

Several researchers have investigated the synergistic behavior and applications of catanionic (i.e., mixed systems of cationic and anionic surfactants) [26, 27, 28, 29]. The development of a catanionic surfactant began with Kaler's publication [30]. Since then, great efforts have been made to study the mixed behavior of catanionic surfactants. The systems consisting of single cationic surfactants mixed with anionic ones were described [31, 32]. It is worth mentioning that catanionic surfactants show a synergistic effect, and exhibit lower critical micelle concentration, faster diffusion rates, and superior wetting capability compared to single surfactants.

The antimicrobial synergistic effects of CTAC and SCO mixes have been investigated, to do that a mix of 1% CTAC with three concentrations of SCO (1%, 2.5%, and 5%) has been improved. The mixes containing 1% CTAC and 5% SCO have the highest inhibition zones: 17.48±0.02mm for S. aureus, 18.59±0.13mm for B. subtilis, 14.71±0.03mm for E. coli, and 12.39±0.05mm for P. aeruginosa as shown in Table 5 and Figure 1. A one-way ANOVA showed a statistically significant difference in inhibition zones for *B. subtilis* among the three SCO+CTAC concentrations, F (27.25), p = 0.001. Post hoc analysis using Tukey's HSD indicated that 5%SCO+1% CTAC mix scored significantly higher than the two mixes.

The synergistic antibacterial behavior of the CTAC and SCO mixture is evident from the results. The higher the concentration of the anionic activator, the stronger the synergistic behavior. This may be attributed to the ability of the anionic activator to disintegrate the outer membrane of bacteria, which facilitates the penetration of the cationic activator into the bacterial cells.

Despite the synergistic improvement of the antibacterial efficiency with increasing the SCO doses in the CTAC-SCO mixes. However, the increase did not correspond to the doubling of the SCO doses. For example, when moving from mix1 (1% CTAC + 1% SCO) to mix3 (1% CTAC + 5% SCO), Staphylococcus Aureus showed an increase in the diameter of the inhibition zone of 1.92 mm, while Bacillus Subtilis was 1.37mm. For Gram-negative species Escherichia coli and Pseudomonas aeruginosa, the increases were relatively slight 0.31 mm and 0.3 mm, respectively . This nonlinear behavior may be attributed to micelle saturation, which reduces the availability of free CTAC molecules. Therefore, despite the apparent synergy, optimizing the concentration ratios is necessary to achieve a balance between physical and biological properties [33].

The performance of CTAC and SCO mixtures on Gram-positive showed more antibacterial efficacy than Gram-negative. The bacterial sensitivity detected to be decreased in the following order: Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa). This is due to differences in the composition and permeability of the cell wall of the bacterial strains studied. Gram-positive bacteria have a simple cell wall structure and lack an outer membrane, allowing easier penetration of CTAC, which explains the stronger response of Gram-positive bacteria. In contrast, Gram-negative bacteria have an outer membrane that constitutes a significant barrier to CTAC activity, explaining their relatively low inhibitory yield. Although Gram-positive bacteria were more affected, Gram-negative bacteria also showed susceptibility, despite their inherent resistance**.**

F. TTC Reduction Assay and Visual Indicators of Antibacterial Activity

A method for detecting bacterial susceptibility by TTC reduction reaction provided visual confirmation of the antibacterial performance of the cement paste mixes. In this assay, in the presence of bacteria, TTC was reduced to red formazan, whereas faded or colorless wells suggest metabolic inhibition and, therefore, effective antibacterial action. The presence of DMSO and a blank (control samples) resulted in a heavy red color from the transformed formazan in growth media, confirming high bacterial viability in the absence of antimicrobial agents as shown in Figure 2.

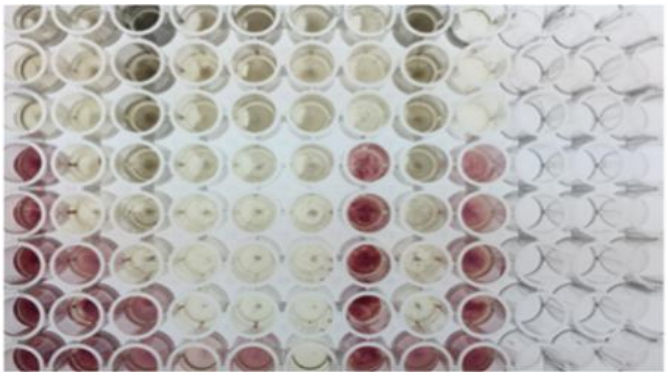


Fig. 2. Growth of four species bacteria in Microbroth Dilution (MBD) with Tetrazolium Chloride (TTC).

The reduction in color intensity until appearing nearly colorless was observed with the increasing of CTAC concentration and its mixtures with saponified cooking oil (SCO).This observation is consistent with the inhibition zone data, confirming the antibacterial efficacy of the tested mixtures and also confirming the synergistic activity of the CTAC and SCO mixture.

V. LIMITATIONS

This study primarily focused on evaluating the antibacterial activity of cement pastes using zone of inhibition measurements and TTC reduction Assay. While these results indicate enhanced antimicrobial performance, the interpretation of synergistic effects remains qualitative. Due to the absence of minimum inhibitory concentration (MIC) data the observed synergy is considered hypothetical rather than confirmed. Additionally, *mechanical property tests, such as compressive strength and setting time, were not included in this study.*

However, these properties were evaluated in a complementary study currently under separate publication and were therefore not repeated here to avoid data overlap. Future studies should include both comprehensive mechanical testing and MIC-based synergy assessment to fully validate the functional performance and practical applicability of the modified cementitious materials.

VI. Conclusion

This study demonstrated the antibacterial effectiveness of incorporating Cetrimonium Chloride (CTAC) and saponified cooking oil (SCO) into cement paste as well as the synergic effect of their formulations as a strategy to enhance antibacterial performance. The obtained results reveal the notable dose-dependent antibacterial activity of CTAC, with higher concentrations yielding larger zones of inhibition activity against both Gram-positive and Gram-negative bacteria. The mixes of SCO with 1% CTAC, showed a synergistic enhancement in antibacterial activity based on qualitative data. While SCO and CTAC demonstrated effective antibacterial enhancement in cementitious materials, their potential environmental impact due to leaching should not be overlooked. In particular, quaternary ammonium compounds like CTAC are associated with aquatic toxicity. Further research assessing the leachability and ecological risks of these additives is recommended to ensure safe long-term application in construction materials.

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