Antibacterial Properties of Cement Pastes with Addition of Lithium Hydroxide, Cupric Oxide, and Titanium Dioxide

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Abstract

In order to provide improved functionalities to concrete, new materials are being studied as antibacterial additions. This could result in a reduction in bacteriological hazards bonded to the surfaces of concrete structures, reducing the requirement for cleaning and chloride use. Therefore, this study evaluates the antibacterial action, and the compressive and flexural strength of cement pastes made with different water/cementitious material ratios (0.35, 0.40, and 0.45) and addition percentages of lithium hydroxide (LiOH), cupric oxide (CuO), and titanium dioxide (TiO2) (1%, 3%, 5%, and 10% by weight) separately on Escherichia coli. Antibacterial activity was assessed using inhibition zone measurements, optical density (OD at 600 nm), and colony-forming unit (CFU) counts after exposure to E. coli. Mechanical performance was evaluated through flexural and compressive strength testing at 7 and 28 days. Results show that LiOH decreases the pastes' strength while producing the highest antibacterial effect due to its high reactivity and pore-inducing behavior; CuO demonstrated the lowest antibacterial effect due to agglomeration issues but increased strength due to its inert nature and filler effect; and TiO2 had an intermediate antibacterial effect with less strength loss than LiOH, although its antibacterial property was not activated by UV require to its photocatalytic mechanism. Also, it was found notable discrepancies between antibacterial assessment methods, highlighting the need for a multi-technique approach to reliably evaluate antimicrobial performance in cement-based materials. Furthermore, the dispersion of microparticles within the paste significantly influenced both antibacterial efficacy and strength development, suggesting that proper mix design and potential use of dispersion aids are critical for industrial applications. As a result, it was concluded that the three compounds could be used as potential additions to concrete to produce an antibacterial effect. Nevertheless, CuO and TiO2 are highly dependent on the dispersion grade, whereas lithium salts are among the best alternatives due to their soluble potential.

Keywords: Antibacterial concrete, lithium hydroxide, cupric oxide, titanium dioxide, *Escherichia coli*.

1. Introduction

Concrete is the most-used building material worldwide due to its wide range of applications (roads, foundations, civil works, sewer systems, marine structures, bridges, high-rise buildings, etc.) (Noeiaghaei et al., 2017). According to the literature, the broad use of concrete is due to its mechanical resistance, durability, and low cost; however, its high environmental impact requires the constant use of new materials that can improve its microstructure and, therefore, its performance (Metha & Monteiro, 2006). Nevertheless, today it is thought that concrete can offer greater functionality when used for non-structural applications such as CO_2 capture, water decontamination, or heat isolation among others. From these new applications, the question of whether concrete can be used as an antibacterial material arises.

One of the ways to evaluate antibacterial materials, e.g. concrete, is by using *Escherichia coli* (ASTM E2149-20). In addition, *E.coli* is a bacterium used as an indicator of fecal contamination in the environment as it is part of the coliforms present in wastewater (Khan & Gupta, 2020). Therefore, when evaluating the antibacterial properties of a concrete, using *E. coli* is a good indicator. Especially from the point of view, that concrete could be used in wastewater pipelines or in the design of wastewater treatment plants to contribute to the decrease of coliforms such as *E. coli* in wastewater. Pathogenic strains can cause extraintestinal illness, leading to large-scale poisoning outbreaks if transmitted to humans (Maule, 2000; Wilks, Michels, Keevil, 2005). One of the main complexities of *E. coli* is the great ease and rapidity with which it is transmitted through contaminated surfaces (Griffin & Tauxe, 1991; Tilden et al., 1996; Wilks et al., 2005); for these reasons, *E. coli* has been extensively investigated to evaluate the antibacterial effect of different materials added to concrete (Qiu et al. 2020). *E. coli* is present in wastewater, and one of the processes to reduce its presence is the use of chlorine after wastewater treatment, but this generates an increase in operating costs, also traces of chlorine are transmitted to the environment, which can generate an accumulation of chlorinated compounds in the environment (Huang et al. 2014), so it is important to look for other materials that can generate an antibacterial effect and do not generate an impact on the environment.

New technologies are aimed at efficiently controlling the growth of microorganisms, with antibacterial concretes offering alternatives to improved photocatalytic and hydrophobic functionalities. While zinc-based materials have often been used to produce antibacterial concretes (Singh et al., 2018), elements and compounds such as copper, lithium, or titanium can be evaluated to develop new and more effective solutions. Copper is usually one of the first alternatives to be used as an antibacterial agent since bacteria die upon contact with it, an effect known as "contact killing" (Grass, Rensing & Solioz, 2011; Nandakumar, Madayiputhiy, Santo & Grass, 2011). During contact with bacteria, copper releases ions from its surface (Molteni, Helge & Solioz, 2010), penetrating the bacterial cell and causing damage at the cytoplasmic membrane level (Hong, Kang, Michels & Gadura, 2012). This increases the oxidative stress

of the bacteria, which, together with oxidative modifications of its proteins, creates functional damage to essential enzymes that finally induce cell death and degradation of bacterial DNA (Prado, Vidal & Durán, 2012). The effect is maintained over time, inhibiting surface colonization, limiting bacterial spread, and reducing the development of biofilms (Rogers et al., 1994; Kielemoes & Verstraete, 2001; Schmidt et al., 2012; Colin et al., 2018). With the use of copper in a construction area, Haile, Nakhla, Allouche and Vaidya (2010) performed tests on concrete pipes electrochemically coated with nano-copper oxide, which concluded by establishing a minimum concentration limit to inhibit bacteria between 2.3 and 2.6 mg of Cu per mg of dry cell weight. On the other hand, using 5% and 10% copper sulfate pentahydrate ($CuSO_4$-$5H_2O$) by weight of Portland cement, Grengg et al. (2021) observed no difference in antibacterial properties. It is worth noting that sulfate-rich materials induce ettringite risk in concrete (Collepardi, 2001); therefore, they should be avoided. Similarly, lithium has been studied as an antibacterial, antiviral, and antiparasitic agent (Lieb, 2007). According to the literature, the antibacterial activity of lithium occurs at high-purity concentrations (Tian et al., 2020). Its antibacterial action, similar to the proposed removal of copper, begins with the entry of lithium ions that cause the cell wall's destruction, inducing desertion and subsequent dehydration of the cytoplasmic contents (Keikhosravani et al., 2021). In addition, the influx of these ions can cause oxidative DNA damage and the peroxidation of bacteria (Tian et al., 2020). This effect has been widely studied in the healthcare field, with observed benefits in the treatment of psychiatric conditions, such as stabilizing mood in patients with mania disorders (Cade, 1949; Noack & Trautner, 1951; Machado-Vieira, Manji & Zarate, 2009; Malhi & Geddes, 2014). At the same time, Ali et al. (2021) achieved antibacterial effects in dental use by eliminating biofilms. However, using lithium as an antibacterial agent in concrete has yet to be adequately explored. Finally, titanium dioxide (TiO_2) is a widely studied element for environmental decontamination and the fight against microorganisms and comes in three crystalline forms: rutile, anatase, and brookite (Allen et al., 2018). Its antibacterial properties are attributed to its photo sterilization effect on the oxidation of complex proteins and inhibition of enzymatic functions of bacterial cells that cause cell death (Hamal et al., 2010). S. Roy, Parveen, Koppalkar, and Prasad (2010) demonstrated that anatase has an antimicrobial effect even on antibiotic-resistant bacteria such as *Staphylococcus aureus.* Therefore, Ganji, Allahverdi, Naeimpoor, and Mahinroosta (2015)Ganji, Allahverdi, Naeimpoor & Mahinroosta (2015) conducted a study to determine the photocatalytic effect of nano-TiO_2 loaded cement, observing a decrease in colony-forming units by adding 5% by weight of TiO_2 to the cement.

Nowadays, research on antibacterial additions to concrete commonly involves nanoparticles. However, different authors have questioned the use of nanoparticles for applications where concrete can release the particles into the environment, raising health concerns (Oliveira et al., 2019; Mohajerani et al., 2019). As an example of this, copper nanoparticles can penetrate human cells and organs, causing neurotoxity (Ameh & Sayes, 2019; Mohajerani et al., 2019). Therefore, if released from concrete and transferred to people or the environment through direct contact, these nanoparticles could cause a sanitary emergency. Additionally, nanoparticles present an elevated cost and a high tendency to clump, limiting their application during concrete mixing at the industrial scale, losing effectiveness, and requiring larger dosages than in laboratory studies (Khaloo et al., 2016; Mohajerani et al., 2019). These drawbacks are currently prompting researchers to return to studying microparticles instead of nanoparticles. However, new studies are required on microparticles of CuO and TiO_2 as antibacterial additions in cement-based materials, even with the known potential of the copper and titanium. On the other hand, the soluble nature of lithium salts offers an opportunity to avoid the issue of particle size. However, in the literature, most of the cases involved the study of lithium in a pure state or as carbonate for effectiveness or cost, respectively. Currently, the growing lithium mining industry encourages the study of different forms, especially with the knowledge that in this case, there is an intrinsic reaction between the cement paste and the addition. However, there are no studies on the use of LiOH, particularly in large dosages, as an antibacterial agent in cement pastes. Therefore, based on the literature review summarized above, this research seeks to determine and compare antibacterial performance of cement paste by using micro CuO, micro TiO_2, and soluble LiOH under the same conditions to determine which material presents the greatest potential. Moreover, most of the previous studies do not compare different antibacterial analyses, creating a gap regarding which technique (inhibition area, optical density, or colony-forming units) is best for the study of cement pastes. While the results confirm a proper antibacterial effect of cement pastes, their performance is varied. CuO raises mechanical strength but has a lower effect on the growth of E. Coli, LiOH has a high antibacterial effect but can sharply impact mechanical strength, and TiO_2 results in an intermediate reaction between CuO and LiOH. This work allows progress to be made in the study of lithium salts in concretes subject to the presence of bacteria that can deteriorate the material or pose danger to human health.

2. Materials and methods

2.1. Raw materials and mixing procedure

Cement pastes were made using high-strength cement, according to NCh 148. Additionally, the cement pastes included soluble lithium hydroxide monohydrate of industrial grade provided by SQM (LiOH, 99%) and microparticles of cupric oxide (CuO, 99.9% of purity) and titanium dioxide (TiO_2, 99.9% of purity) with food grade. The chemical and morphological composition of each material was determined using Energy-dispersive X-ray spectroscopy (FESEM-EDX) with a Quanta FEG-250 microscope; each sample was coated with 4 nm of gold and later mounted on a holder. The particle sizes of CuO, LiOH, and TiO_2 were obtained from SEM images.

The experimental design was formulated using a factorial method for each material, including, as independent variables, the water-cementitious material (w/cm: 0.35, 0.40, and 0.45) and the percentage of cement replacement in weight by each addition (1%, 3%, 5%, and 10%). In each material, it was considered that the additions could react with the cement paste to keep the water-cement ratio constant according to the experimental matrix. Additionally, three control mixes, one for each w/cm ratio, were generated to compare the responses. The experimental matrix can be observed in Table 1. To ensure proper workability, Viscocrete 6000 plasticizer (Sika, Chile) was added to the mixes in the function of the w/cm ratio to avoid porosity issues. Regarding the mixing procedure of the cement pastes, LiOH was previously dissolved in the water of the mixes, while TiO_2 and CuO were dry premixed with the cement powder. A standard Hobart paddle mixer was used. The process began when the plasticizer was dissolved in the water (with LiOH, if necessary); the cementitious material was then poured. Plasticizer was used in dosages of 0.6, 0.5, and 0.4% of the cement weigth for the w/c ratios of 0.35, 0.40, and 0.45, respectively to get similar workability and void miss vibration issues. This action took no more than 15 s, mixing by 30 s at 146 rpm; then, the mixing bowl was cleaned, and 90 s of additional mixing at 281 rpm was performed. After the mixing, six 40x40x160 mm prisms, three 20x20x20 mm cubes, and three 10x10x5 mm pills were cast. Subsequently, the setting time was developed according to ASTM C 191-19 using a Vicat apparatus to determine the mixture's workability.

Tab. 1. Experimental Matrix.

Mix	Addition	w/c	% as SCM	Mix	Addition	w/c	% as SCM
C-0.35-0	-	0.35	0	**CuO-0.40-5**	CuO	0.40	5
C-0.40-0	-	0.40	0	**CuO-0.40-10**	CuO	0.40	10
C-0.45-0	-	0.45	0	**CuO-0.45-1**	CuO	0.45	1
LiOH-0.35-1	LiOH	0.35	1	**CuO-0.45-3**	CuO	0.45	3
LiOH-0.35-3	LiOH	0.35	3	**CuO-0.45-5**	CuO	0.45	5
LiOH-0.35-5	LiOH	0.35	5	**CuO-0.45-10**	CuO	0.45	10
LiOH-0.35-10	LiOH	0.35	10	**TiO_2-0.35-1**	TiO_2	0.35	1
LiOH-0.40-1	LiOH	0.40	1	**TiO_2-0.35-3**	TiO_2	0.35	3
LiOH-0.40-3	LiOH	0.40	3	**TiO_2-0.35-5**	TiO_2	0.35	5
LiOH-0.40-5	LiOH	0.40	5	**TiO_2-0.35-10**	TiO_2	0.35	10
LiOH-0.40-10	LiOH	0.40	10	**TiO_2-0.40-1**	TiO_2	0.40	1
LiOH-0.45-1	LiOH	0.45	1	**TiO_2-0.40-3**	TiO_2	0.40	3
LiOH-0.45-3	LiOH	0.45	3	**TiO_2-0.40-5**	TiO_2	0.40	5
LiOH-0.45-5	LiOH	0.45	5	**TiO_2-0.40-10**	TiO_2	0.40	10
LiOH-0.45-10	LiOH	0.45	10	**TiO_2-0.45-1**	TiO_2	0.45	1
LiOH-0.35-1	LiOH	0.35	1	**TiO_2-0.45-3**	TiO_2	0.45	3
CuO-0.35-1	CuO	0.35	1	**TiO_2-0.45-5**	TiO_2	0.45	5
CuO-0.35-3	CuO	0.35	3	**TiO_2-0.45-10**	TiO_2	0.45	10
CuO-0.35-5	CuO	0.35	5	**TiO_2-0.35-1**	TiO_2	0.35	1
CuO-0.35-10	CuO	0.35	10	**TiO_2-0.35-3**	TiO_2	0.35	3
CuO-0.40-1	CuO	0.40	1	**TiO_2-0.35-5**	TiO_2	0.35	5
CuO-0.40-3	CuO	0.40	3	**TiO_2-0.35-10**	TiO_2	0.35	10

2.2. Mechanical strength

To evaluate the mechanical strength of the cement pastes, three 40x40x160 mm samples were tested at 7 days and another three samples at 28 days, according to the ASTM C348. Flexural strength was measured by loading the samples in the center with a load rate of 5 Kgf/s, and the compressive strength was obtained from the halves loaded at a 15 Kgf/cm^2/s rate. After the compression testing, core fragments from samples with a w/c ratio of 0.45 and the highest addition percentage were collected and milled for SEM analysis.

2.3. Antibacterial effect

The 10x10x5 mm samples were placed in Petri dishes containing LB agar and a culture of *Escherichia coli* (OD_{600} 0.1), which was spread on the surface of the agar. The plates with the concrete samples were incubated at 37 ºC for 18 h. At the end of the incubation period, the antibacterial properties of the samples were evaluated. The analysis was performed by measuring the inhibition area (the sector around the concrete sample without bacterial growth), as seen in Fig. 1A.

At the same time, the three 20x20x20 mm samples were placed in an *E. coli* solution for 16 h with constant oscillation (130 rpm). Afterward, the antibacterial effect of the samples was measured by optical density (600 nm, see Fig. 1B) and by counting the colony forming unit (CFU/mL), as seen in Fig. 1C.

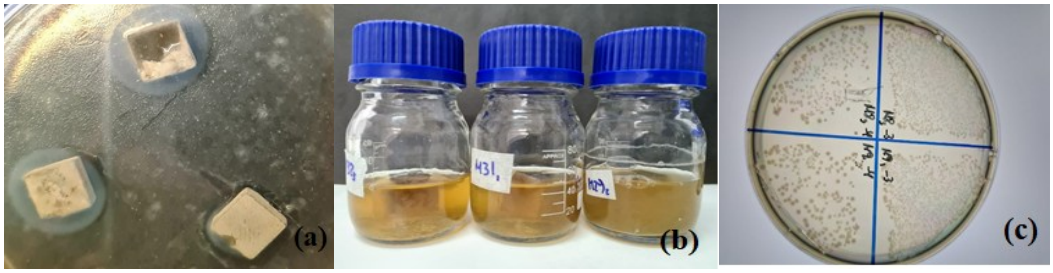


Fig. 1. Sample analysis of antibacterial effect. a) Inhibition area assay, b) Optical density, c) Colony forming unit (CFU/mL).

3. Results

3.1. Materials characterization

Tab. 2 shows the chemical composition according to the SEM-EDX results of each material, and Fig. 2 shows the morphology of the materials. EDX could not measure LiOH because the equipment does not measure Li; however, the materials conform to LiOH chemical composition. As shown in Fig. 2C, TiO_2 has an average particle diameter of 1.6 µm. On the other hand, CuO forms 100µm cumulus in average, formed by microparticles from 10 to 30 µm (Measured from a 500x SEM image). Lithium particle size was not possible to measure due to the irregularity of the crystals. However, they can be described as soluble cumulus between 0.1-5 µm (Fig. 2d).

Tab. 2. Chemical composition of extra cement. CuO, LiOH, and TiO2

	O	**Al**	**Si**	**S**	**Ca**	**Cu**	**Li**	**Ti**	**Total**
Extra cement	31.39	0.71	11.56	0.78	55.57	-	-	-	100.00
CuO	8.76	-	-	-	-	91.24	-	-	100.00
TiO2	50.35	-	-	-	-	-	-	49.65	100.00

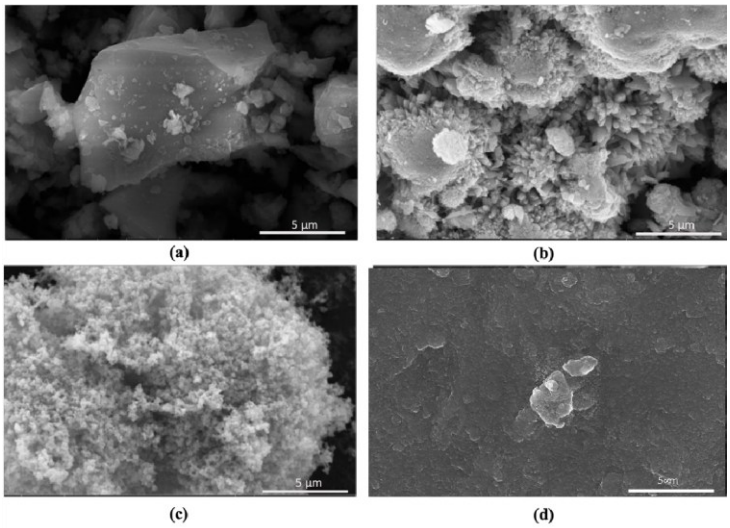


Fig. 2: Scanning electron microscopy (SEM): a) cement, b) CuO, c) TiO_2, and d) LiOH.

3.2. Setting time

Fig. 3 shows the setting time of mixes with a 0.40 w/cm ratio and 10% addition for each material. From the results, it can be observed that LiOH sharply accelerates the set (~90%). However, in the case of CuO, despite having a lower setting time than the control sample, the reduction was ~15%. Finally, TiO_2 delayed the setting time by ~30%.

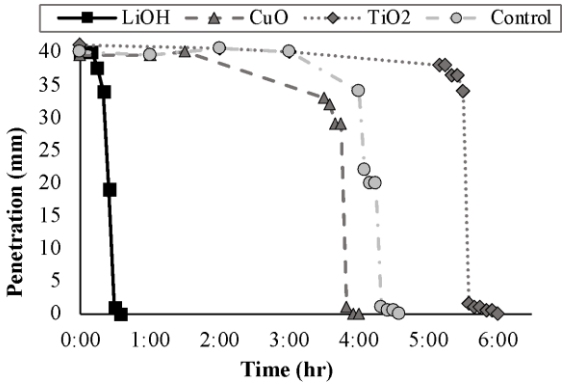


Fig. 3. Setting time of mixes for each material (LiOH: lithium hydroxide; CuO: cupric oxide; TiO_2: titanium dioxide).

3.3. Flexural strength

Fig. 4, Fig. 5, and Fig. 6 present the results of the flexural strength using LiOH, CuO, and TiO2, respectively, at 7 days (a) and 28 days (b). Results show that the 7-day strength is increased (~20%) in LiOH mixes with dosages lower than 3% and severely decreased (~60% less) with dosages of 10%; at 28 days, results show less improvement with low LiOH addition, with more significant strength losses at higher addition percentages. On the other hand, copper mixes offered an improvement of at least 10% with dosages ≥3% at 7 days, without a significant difference in the function of the percentage of addition; however, at 28 days, the mixes provided an improvement of ~18% in combinations with 1% of CuO, resulting in a decrement in flexural strength from dosages ≥5%. Finally, TiO2 mixes presented a rise in strength at 7 days with 1% of addition (~50%), with this improvement reducing gradually until 10% of addition. Also, 0.45 w/cm mixes gave the highest improvement in the control mixes at 7 days.

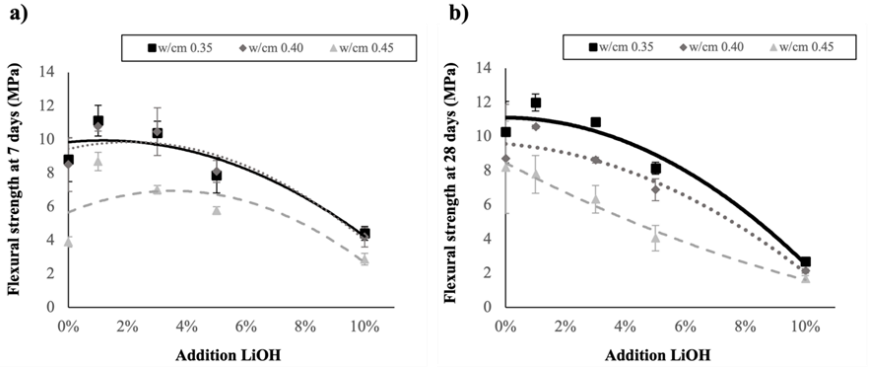


Fig. 4: Flexural strength of LiOH cement pastes. a) at 7 days, b) at 28 days.

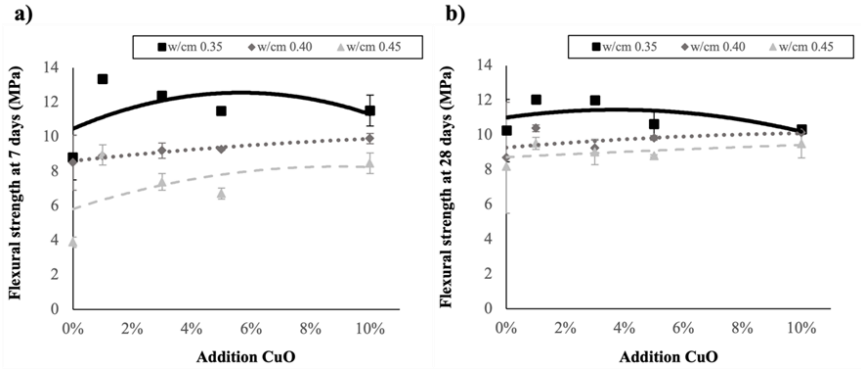


Fig. 5: Flexural strength of CuO cement pastes. a) at 7 days, b) at 28 days.

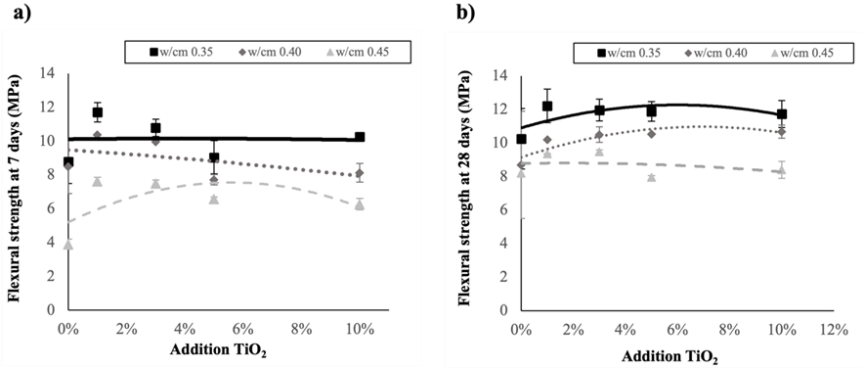


Fig. 6: Flexural strength of TiO_2 cement pastes. a) at 7 days, b) at 28 days.

3.4. Compressive strength

Fig. 7, Fig. 8 and Fig. 9 show at 7 days (a) and 28 days (b) the compressive strength of the three control samples and the mixtures made with LiOH, CuO, and TiO_2. It can be observed that, in all the mixtures, there is an increase in compressive strength over time; however, the mix with LiOH has the lowest strength and is the most affected by the increase in the percentage of addition, while the samples with TiO_2 and CuO vary more significantly depending on their w/cm ratio. In fact, CuO mixes with low w/cm have the highest improvement at 28 days but with a low difference in function of the addition dosage. It should be noted that, the pastes with CuO have the highest compressive strengths. On the other hand, the variation between the tests performed on the mixtures with TiO_2 at 28 days does not exceed 15%, in contrast to the tests performed at 7 days, while the pastes with LiOH addition are close to 20%.

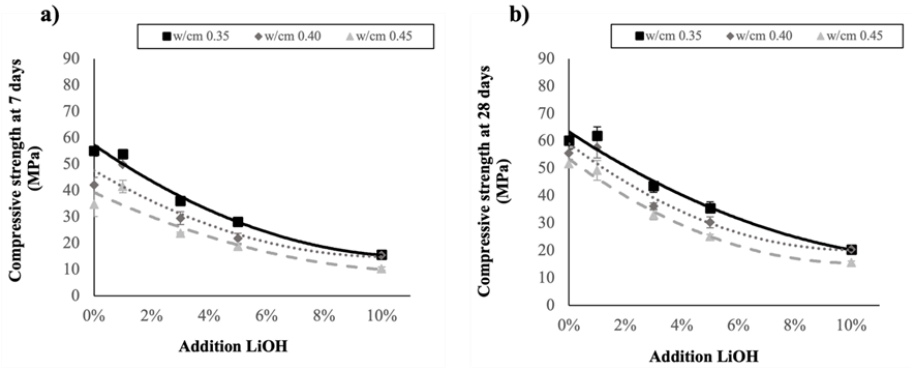


Fig. 7. Compressive strength of LiOH cement pastes. a) at 7 days, b) at 28 days.

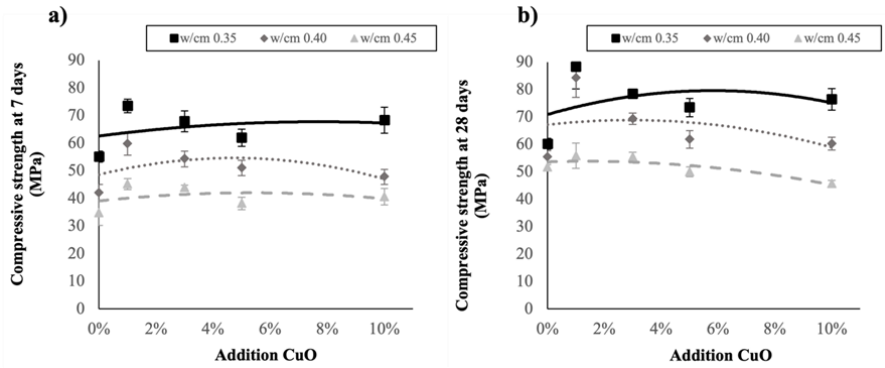


Fig. 8. Compressive strength of CuO cement pastes. a) at 7 days, b) at 28 days.

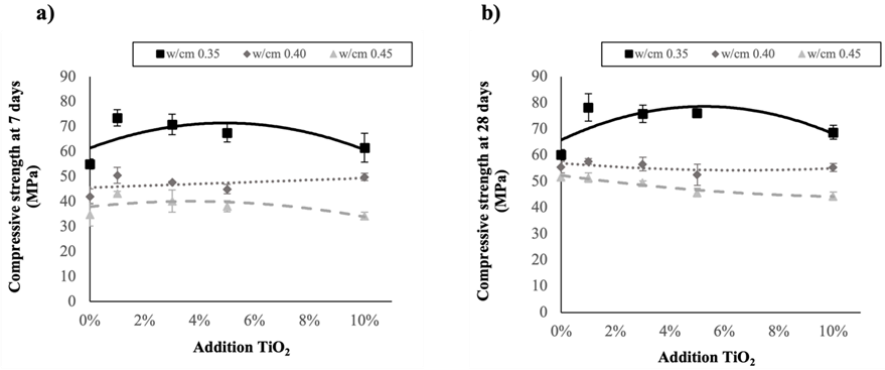
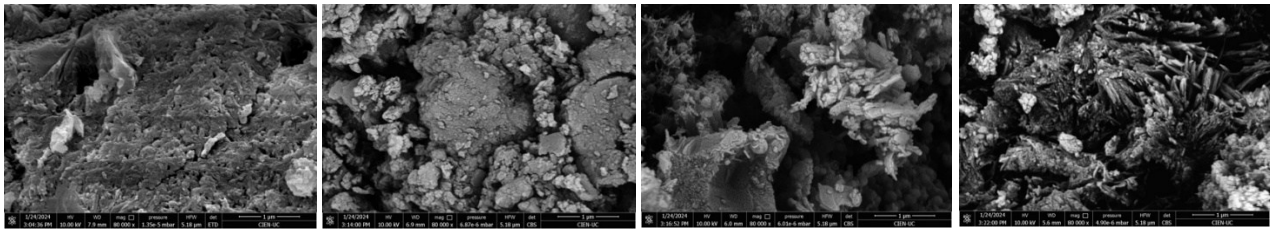


Fig. 9. Compressive strength of TiO_2 cement pastes. a) at 7 days, b) at 28 days.

4. SEM of cement pastes

Scanning Electron Microscopy (SEM) analysis of Fig. reveals that the inclusion of copper-based additives in cement pastes tends to result in discrete, undissolved particles that act primarily as inert fillers. These particles do not significantly engage in the hydration process but may influence durability and porosity depending on their chemical for (Ying & Ye, 2024). In contrast, lithium hydroxide (LiOH) is fully dissolved in the pore solution explaining why it alters the cement paste's morphology, setting times, and strength at higher dosages due to interference with calcium-silicate-hydrate (C–S–H) formation (Deng et al., 2014).Titanium dioxide (TiO_2) nanoparticles exhibit a more interactive behavior; they refine the pore structure and act as nucleation sites, promoting a denser and more compact matrix that improves mechanical performance and reduces porosity (Hu et al., 2024; Li et al., 2017). These distinct microstructural effects reflect the varying degrees of chemical interaction, with lithium exhibiting the strongest chemical reactivity, especially with calcium silicates—often at the cost of structural integrity in higher dosages.



(a) (b) (c) (d)

Fig. 10: SEM images of w/c 0.45 cement pastes: (a) control, (b) 10% of LiOH, (c) 10% of CuO, and (d) 10% of TiO_2

4.1. Inhibition area

Fig. 11 shows the inhibition areas of the 10x10x5 mm tablets that were made. As shown, the efficacy of LiOH increases as the water-cement ratio increases, with greater effectiveness between 3% and 5 %. On the other hand, CuO loses its antibacterial capacity with an increase in dosage and the w/c ratio. Mainly, this can be attributed to higher doses generating agglomeration of the particles that were only dry mixed, achieving a lower homogenization of CuO within the paste, regardless of the addition of a more significant percentage of CuO. In Fig. 11, it also can be observed that, the tablets with LiOH addition significantly increase bactericidal inhibition for the ratio a/cm 0.45. It is mainly for the concentrations of 1% and 5%; in comparison, the tablets with CuO for the same ratio a/cm and concentrations of 3% and 5% have an area size equal to 100 (the same size of the tablet). Hence, the bactericidal inhibition of the tablets is equal to zero. On the other hand, the tablets with TiO_2 addition showed lower efficacy as the w/cm ratio increased; however, no significant variation was observed based on the percentage of addition within the same w/cm ratio.

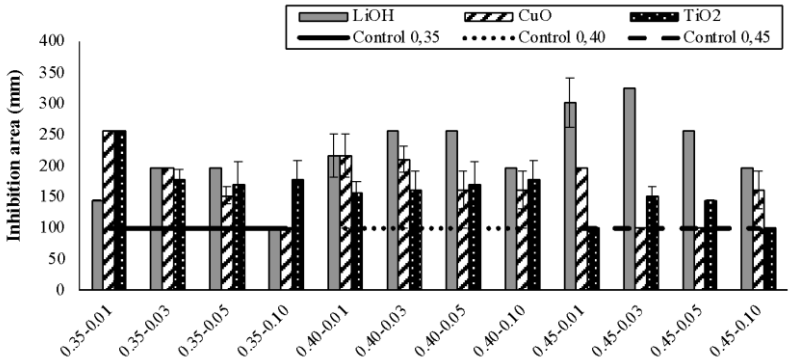


Fig. 11. Inhibition area of cement pastes at 28 days.

4.2. Optical density

Fig. 12 shows the results of the optical density (OD) test. As one can observe, LiOH generated a decrease in the optical density, with the highest value observed for doses of 10% LiOH. On the other hand, an increase in antibacterial capacity was also observed in mixtures with a high w/cm ratio. This can be explained by the fact that higher w/cm ratios have higher permeability, achieving a more significant paste-bacteria interaction during the agitation period. CuO achieved a low antibacterial performance, acting satisfactorily in low w/cm ratio mixtures. Similar to the previous results, low doses of CuO achieved the highest inhibitions in general

terms. Finally, TiO_2 achieved a decrease in optical density without significant differences in terms of w/cm ratio or percentage of addition. It should also be noted that a white layer on the surface of the medium was present in the mixtures with TiO_2, indicating that during agitation, the compound could account for part of the turbidity, making this methodology less certain.

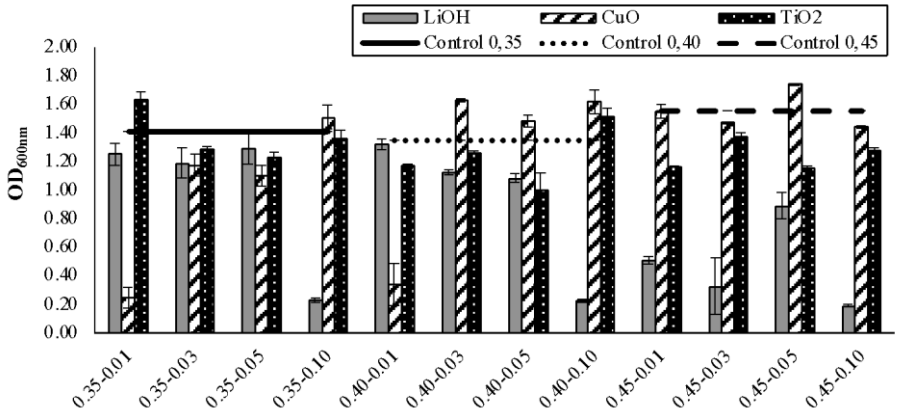


Fig. 12. Optical density of cement pastes at 28 days.

4.3. Colony-forming units

Fig. 13 shows the CFU count after the agitation process for OD measurement. In this case, a decrease in CFU by LiOH is observed, indicating better results at higher w/cm ratios and LiOH concentrations. It should be noted that, despite the CFU measurements being repeated in buckets with 10% LiOH, the count still resulted in zero. CuO presented results similar to those of OD and inhibition area, showing a lower antibacterial resistance of the paste at higher w/cm ratios and CuO concentrations. Finally, TiO_2 created an inhibitory action but maintained a behavior with a neutral trend in terms of w/cm ratio and addition percentage.

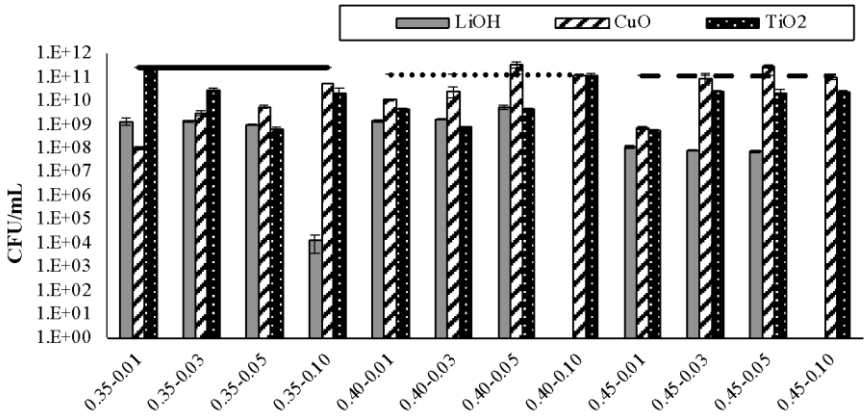


Fig. 13. Colony-forming units (CFU/mL) of cement pastes at 28 days.

5. Discussion

To address the effect on the mechanical strength of cement pastes with lithium, CuO, and TiO_2 additions, the 28-day flexural and compressive strength of each element was normalized by its control sample and cement percentage. Results show that mixes with lower w/cm ratio loss efficiency in terms of flexural strength, particularly mixes with lithium, were the most affected. On the other hand, mixes with TiO_2 at higher w/cm ratio improve their strength, mainly for the percentages of 3% and 5%, while for the pastes with CuO, they can obtain an adequate strength ratio, excepting mixes with 10% of each addition. Regarding the compressive strength at 28 days, CuO presents higher ratios, even in a ~40% at 1% of addition, but its effect is decreased both the w/cm ratio and the percentage of addition. TiO_2 mixes presented a similar behavior but with a lower improvement; finally, LiOH highly decreased the ratio in function of the LiOH percentage. The strength loss by Lithium is explained because it has a high affinity with the calcium hidroxyde, affecting the formation and stability of CSH (Fang & Zhang, 2021; Mitchell et al., 2004). Besides, LiOH generate pores in the fresh cement paste, reducing the strength (Fang & Zhang, 2021; He et al., 2021). On the other hand, CuO has low chemical reactivity with the cement paste's microstructure, resulting in inert cupper microparticles entrapped in the cement paste. These particles present a high hardness resulting in a filler effect that increases the mechanical strength of the pastes, especially under compression. These results vary from Finally, while nano TiO2 has shown improvement in the cement pastes microstructure by favor the hydration and homogenization of the CSH (Shaaban et al., 2020), micro TiO_2 present opposite results. High dosages of micro TiO_2 reduce the strength but still is not clear because non-hydrated particles act as a low-strength filler, because reduce the workability, or because it affects the CSH microstructure negatively.

Additionally, from the results, it is clear that LiOH, CuO, and TiO_2 presented an antibacterial effect. LiOH produced higher results, with a difference between the inhibition area test and the OD and CFU/mL measures. At the same time, it was found that higher CuO additions generated lower antibacterial effects than noted in the literature; however, different authors indicated that particle size and higher dosages reduce the dispersion (Parveen, Rana & Fangueiro, 2013), explaining the results. As a result, additives that improve the homogenization or sonication methods are necessary to improve the CuO benefits. Finally, TiO_2 reduced the bacteria counts; however, its effect was limited. According to the literature, the response of TiO_2 could be enhanced using UV light because the effect of the compound is based on photocatalysis (Li-Fen, Barford, King Lun & Grace, 2007). As Fig. 11, Fig. 12, and Fig. 13 demonstrate, the antibacterial effect

of cement pastes must be carefully addressed due to disperse results between the inhibition area, OD, and CFU. As the added materials are distributed in the cementitious matrix, they tend to present lower diameters in the inhibition area. Due to oscillation, particles detached from the cubes can affect the OD test. These highlights using materials harmless to human health, as in this case, where food-grade TiO_2 was used. As can be seen in Fig. 15A, the inhibition area did not present any correlation with the OD, indicating R^2 coefficient <0.43 as the best possible fit. On the other hand, OD versus CFU/mL results (Fig. 15B) showed a certain kind of correlation, with a quadratic relationship between both responses with R^2 of 0.82 to LiOH, 0.32 to CuO, and 0.75 to TiO_2. Of the three materials, LiOH presented the highest antibacterial effects as noted in Table 3, which demonstrates the improved ratio results and the confidence level for each material comparison and testing using a paired T-test. While the results are similar in terms of effectiveness, the amount of bacterial reducing prediction varies for each test, especially the inhibition area and optical density of the more conservative methods. In addition, note that it is impossible to ensure a statistical difference between CuO and TiO_2 in all the responses. Finally, it is worth mentioning that antibacterial additions require a proper design to be applied in the concrete industry in order to balance the antibacterial effect and cost of the materials. However, it was possible to achieve antibacterial effects from materials and dosages that do not create a risk to the environment or human health without using special dispersion techniques, favoring potential industrial applications.

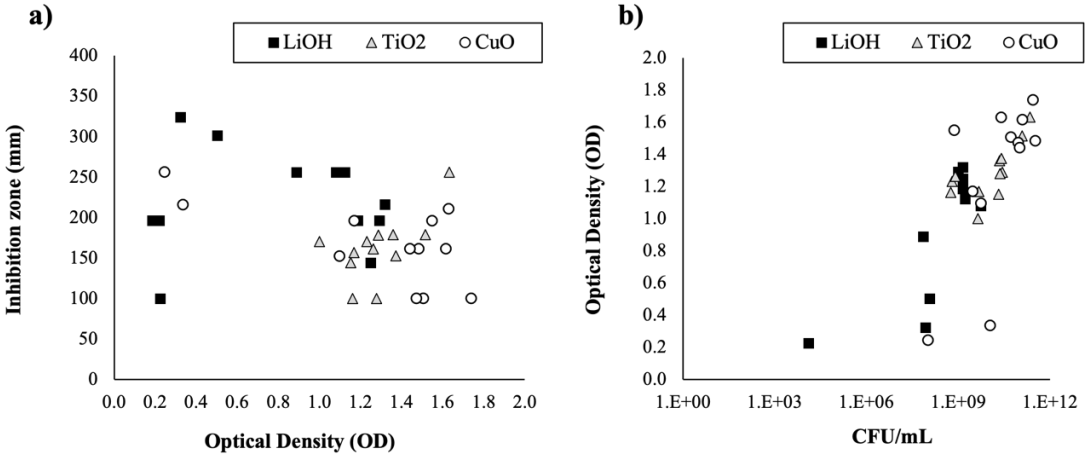


Fig. 14. Antibacterial analysis methods comparison. a) Inhibition area (mm) versus optical density (OD), b) optical density (OD) versus logarithm of CFU/mL.

Tab. 3. Antibacterial average effects ratios. Higher is better for inhibition area; lower is better for optical density and colony-forming units.

		Inhibition area	Optical Density	Colony-forming Units
LiOH/ CuO	Ratio	131%	63%	1%
	confidence level	95%	90%	98%
LiOH/ TiO_2	Ratio	136%	62%	3%
	confidence level	95%	95%	93%
CuO/ TiO_2	Ratio	103%	99%	217%
	confidence level	95%	90%	74%

6. Conclusions

This work contributes to the state of the art by addressing the antibacterial effect of LiOH, CuO, and TiO_2 in cement pastes and studying the effects of each material on strength. In this way, it is possible to advance the development of antibacterial concretes.

The results indicated a high efficacy of the additions, which demonstrate an antibacterial capacity while reducing mechanical strength, which can be recovered by optimizing the mix design. Moreover, the results indicated that the analysis of antibacterial concretes should be carefully developed since traditional inhibition techniques may be discordant. Thus, the results of this research can provide a better interpretation of the use of antibacterial materials in concrete.

From the results, it is possible to establish that LiOH has the highest antibacterial potential among the three materials. However, it dramatically accelerates the setting time and reduces the strength, especially compressive strength, of the cement pastes as the percentage of addition increases. On the other hand, the mixtures with CuO and TiO_2 showed a lower antibacterial effect but also had less impact on the strength of the cement pastes. Even in the case of TiO_2, the materials increased their flexural strengths for w/cm ratios 0.40 and 0.45 and compression for w/c ratio 0.35 and, in the case of the mixtures with CuO, increased the compressive strengths for w/c ratios 0.35 and 0.40. In addition to the intrinsic chemical properties that differentiate LiOH, CuO, and TiO_2, the dispersion obtained and the homogenization achieved during dry premixing could reduce the benefit of the addition of CuO and TiO_2; moreover, TiO_2 may show different results if subjected to UV light.

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