

Explore the Opportunity of Utilizing Some Wastes as an Antibacterial Materials in Construction Applications

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Abstract

Building materials nature is antibacterial materials, but the porosity in this material is considered as proper site to hide bacteria. Construction materials deterioration caused by microorganisms poses a critical issue in the field of sustainability of cementitious infrastructures that face harsh biological conditions. From this fact, antibacterial materials are added to prevent the hazards of bacteria in such materials. The purpose of this work is to utilize antibacterial materials with low cost derived from waste materials that have the same effect as pure materials in killing or inhibiting the growth of bacteria. Three waste materials (copper -based materials, zinc oxide, and eggshell (ESP)) were selected as antibacterial materials. Since the construction materials encountered water and air, the selected bacteria were *Staphylococcus aureus* and *Escherichia coli*. Two approaches were proposed for use in building materials: the first involves adding antibacterial material to the mortar, while the second involves mixing antibacterial material with epoxy that is applied to the surface of the construction material as a coating. Energy dispersive X-ray spectrometry (EDS) analysis was used to confirm the chemical suitability and purity of selected waste-derived antibacterial materials. The tests performed on the physical characteristics support the earlier conclusions related to the antibacterial activities of both systems. The epoxy coatings tested did not possess any porosity or absorption property, thus confirming the assumption that they create a compact and impermeable medium minimizing the risk of moisture penetration and thus can be viewed as a feasible option concerning their antibacterial characteristics. The use of waste materials in cement-based compounds (especially CuO) allowed reducing both porosity and water absorption due to a filling effect preventing free spaces, which interrupts water-borne and air-borne bacteria infiltration. In general, the results confirm that the protective ability of both types of the systems depends not only on the ability of the additive to destroy bacteria but also on the efficiency of the structure obtained. Agar diffusion and colony-forming units (CFU) techniques were used for evaluating the antibacterial activity of waste-derived materials against the

aforementioned bacterial species. Based on the experimental findings, in cementitious composites, all investigated waste-derived antibacterial materials display antibacterial activity. Waste-derived ZnO demonstrates maximum inhibition towards *S. aureus* bacteria, whereas ESP exhibits maximum inhibition toward *E. coli*. Copper-based materials exhibit robust and reliable antibacterial performance against both species. Most importantly, copper based materials are able to inhibit bacterial growth in cementitious composites completely. Moreover, eggshell powder shows excellent antibacterial performance. Epoxy-based specimens demonstrate that all investigated waste-derived antibacterial materials retain their antibacterial ability when used as coating additives, confirming their applicability in surface applications for microbially corrosion protection purposes in construction applications. Cu waste had the highest result.

It can be concluded that both approaches can be applicable, but the waste material should be selected carefully.

Keywords: Antibacterial cement, Antibacterial coating, Sustainable concrete, Waste materials utilization

1. Introduction

Concrete has emerged as the primary construction material owing to its excellent mechanical performance and relatively low cost of manufacture [1]. Its applications cover a wide spectrum of structural elements within various sectors, including residential constructions, transportation networks, marine installations, and subterranean utilities. Owing to continuous increases in the global demand for novel infrastructure in developing countries, there is an urgent need to employ sustainable construction materials with enhanced durability in harsh environments. One of the key challenges in improving concrete durability relates to its vulnerability to biodeterioration processes. MICC (Microbially Induced Concrete Corrosion) refers to biological deterioration, which causes severe damage to concrete structures and leads to the failure of critical infrastructure [2].

This phenomenon occurs due to microbial metabolic activities, particularly by sulfur-oxidizing bacteria such as *Thiobacillus thiooxidans* [3], [4]. In anaerobic conditions, reactions occur between H_2S emitted by sulfate-reducing bacteria and Ca-bearing compounds within the concrete matrix, forming expansive gypsum and ettringite minerals [3], [4]. These processes gradually deteriorate concrete mechanical performance, causing crack formation and spalling. Other microbes responsible for biodeterioration [4].

There is an increasing trend towards studying the antimicrobial behavior of concrete materials because of growing cases of contamination issues and stringent hygiene standards for clinical and food-related applications. Previous research mainly focused on investigating the photocatalytic properties of concrete materials. TiO_2 nanoparticles were extensively explored for this purpose. In their systematic review, Chen & Poon demonstrated that TiO_2 -coated surfaces efficiently degraded organic pollutants and reduced the number of microorganisms upon exposure to UV light [5]. Field trials conducted outdoors confirmed bactericidal properties in natural outdoor settings [6]. Unfortunately, TiO_2 nanoparticles exhibit photocatalytic properties exclusively under UV radiation, limiting their applicability only to external surfaces. Therefore, antimicrobial construction materials based on TiO_2 cannot protect internal elements, submarines, and tunnels from the risk of MIC.

Further developments led to the emergence of antimicrobial concrete with Ag nanoparticle additives. The work by Rai et al. revealed substantial bactericidal activities against Gram-positive and Gram-negative bacteria through various pathways, including membrane disruption, respiratory chain inhibition, and oxidative stress by reactive oxygen species formation [7]. The introduction of Ag nanoparticles to the concrete matrix inhibited bacterial proliferation [8]. Nonetheless, certain restrictions hindered the practical implementation of Ag-based concrete materials. High production costs related to high loading rates, problems related to nanoparticle agglomeration, a lack of information regarding toxicity and ecotoxicology aspects, and the risk of Ag accumulation in aquatic habitats represent some of these constraints [8].

Recently, Cu materials have gained increased attention as affordable substitutes. According to the studies conducted by Grass et al., Cu materials exhibited efficient bactericidal activities by direct contact killing mechanisms against numerous pathogenic bacteria, including *Escherichia coli* and *Enterococcus* [9]. Moreover, Noyce et al. reported effective inhibition of epidemic strains on Cu alloy surfaces under hospital-like conditions [10].

Interest in ZnO nanoparticles promoted the investigation of their application in concrete. Sirelkhatim et al. summarized the antibacterial mechanisms of ZnO nanoparticles involving the formation of reactive oxygen species (ROS), ion liberation, and bacterial wall destruction with potent activities against various pathogenic strains [11], [12].

However, despite abundant literature on this topic, significant gaps remain unfilled. Research efforts rarely focus on sustainable waste-derived sources or evaluate their potential use as functional antimicrobial additives in highly durable construction materials and composites resistant to biodeterioration. This work aims to explore a sustainable waste derived source for developing cost effective antimicrobial additives that improve construction materials' performance.

2 Experimental Work

2.1. Materials

Cementitious materials were Ordinary Portland Cement (OPC) from a local company; the physical properties and chemical composition are shown in Tables 1 and 2. Fine aggregate (sand), which is also supplied from a local company, with grading presented in Table 3.

Table (1): Physical properties of cement.

Property		Iraqi Specification Limits
Physical Properties		
Fineness (m ² /kg)	381	≥ 230
Initial Setting Time (min)	105	≥ 45
Final Setting Time (hr:min)	3:15	≤ 10:00
Soundness by Autoclave (%)	0.03	≤ 0.8
Compressive Strength after 3 days (MPa)	26.6	≥ 15
Compressive Strength after 7 days (MPa)	33.3	≥ 23

Table (2): Chemical composition of cement

Composition		Iraqi Specification Limits
Chemical Properties		
SiO ₂ (%)	17.34	—
Al ₂ O ₃ (%)	4.78	—
Fe ₂ O ₃ (%)	3.10	—
CaO (%)	60.01	—
MgO (%)	2.28	≤ 5
SO ₃ (%)	2.33	≤ 2.8
Insoluble Residue (%)	0.63	≤ 1.5
Loss on Ignition (%)	1.97	≤ 4
C ₃ A (%)	6.74	≤ 3.5
Chloride (%)	0.058	≤ 0.10

Table (3): Grading of fine aggregate

Mesh size (mm)	% Passing by Weight	Specific Limit
4.75	92.3	90-100
2.36	83.7	70-100
1.18	73.9	55-90
0.60	51.8	53-59
0.30	20.2	8-30
0.15	4.6	0-10
Percentage of salts%	0.4	≤0.5

Superplasticizer was added as a chemical admixture, which is a high-range water-reducing admixture (HRWR) that increases workability, reduces the water content, and improves the performance of cementitious materials. The superplasticizer used is (Sika® ViscoCrete®-180 GS), a high-range water-reducing, Retarding, slump-retaining, and superplasticizing admixture for Concrete & Mortar utilizing Sika's ViscoCrete® polycarboxylate polymer technology.

Epoxy resin with properties listed in Table 4. The epoxy, which contains two parts of the resin and the hardener that is used to turn the resin from viscous to hard. The epoxy has strong adhesion quality with moderate cost compared with other polymer resins. Considering it appropriate to offset the cost and coating adhesion properties aspects of the testing criteria.

Table (4): Properties of Epoxy Resin

Property	Value
Material Type	Two-component epoxy resin
Components	Part A + Part B
Mixing Ratio	1:1
Appearance	Crystal clear
Leveling Property	Self-leveling
Yellowing Resistance	Yes
Odor	Low
Flammability	Non-flammable
Working Time	40 min
Complete Curing Time	24 h or more at 25°C

Total Volume	474 mL
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2.1.1. Antibacterial Additives

Different antibacterial materials, including both pure and waste-derived types, were used in this study. The comparison between waste and pure materials allows evaluation of the performance of waste relative to commercially available pure materials. The selected materials are as follows:

- Copper oxide (CuO), Pure and waste copper oxide were selected for use. Table 5 illustrates the standard physical and chemical specifications of pure copper oxide supplied by SRL Company, while copper waste was collected from industrial waste sources.

Table (5): Standard Physical and Chemical Specifications of (CuO) Powder

Property	Specification
Material Name	Copper Oxide Powder
Chemical Formula	CuO
Appearance	Fine black powder
Purity	≥ 99%
Molecular Weight	79.55 g/mol
Density	6.31 g/cm ³
Particle Size	Fine powder (micron grade)
Melting Point	1326 °C (decomposes)
Solubility in Water	Insoluble
pH Value	Neutral to slightly basic
Manufacturer	SRL Company

- Zinc Oxide (ZnO): Zinc oxide was utilized in two different forms, a high-purity ZnO and ZnO derived from industrial waste streams as a low-cost, sustainable alternative. The specifications of the pure ZnO are presented in Table 6 Supplied by SRL Company.

Table (6): Standard Physical and Chemical Specifications of Zinc Oxide (ZnO) Powder

Property	Specification
Material Name	Zinc Oxide Powder
Chemical Formula	ZnO
Appearance	Fine white powder
Purity	≥ 99%
Molecular Weight	81.38 g/mol
Density	5.61 g/cm ³
Particle Size	Fine powder (micron grade)

Melting Point	1975 °C
Solubility in Water	Insoluble
pH Value	Neutral to slightly basic
Manufacturer	SRL Company

- Calcium Carbonate (CaCO_3): CaCO_3 was utilized in two different forms, sustainable natural waste-derived CaCO_3 (Egg Shell Powder) and pure CaCO_3 . Eggshell powder was prepared from waste materials collected from food residues and used as a sustainable alternative source of calcium carbonate. Pure calcium carbonate (CaCO_3) was also used, and its standard specifications are presented in Table 7.

Table (7): Standard Physical and Chemical Specifications of Calcium Carbonate (CaCO_3) Powder Supplied by SRL Company

Property	Specification
Material Name	Calcium Carbonate Powder
Chemical Formula	CaCO_3
Appearance	Fine white powder
Purity	$\geq 99\%$
Molecular Weight	100.09 g/mol
Density	2.71 g/cm ³
Particle Size	Fine powder (micron grade)
Melting Point	Decomposes at ~825 °C
Solubility in Water	Slightly soluble
pH Value	Alkaline
Manufacturer	SRL Company

2.2 Specimens Preparation

2.2.1 Waste Preparation

Waste-derived copper oxide and ZnO materials underwent magnetic separation initially to remove any possible ferrous metals, and then any metal impurities should be eliminated that could be affecting the quality of these materials. Thereafter, manual sieving was conducted to remove coarse debris from the waste materials. An extensively washing using distilled water to eliminate dust, salts, and other contaminants on the surfaces of these materials was carried out. Washing was performed until satisfactory cleanliness was achieved. Upon completion of washing, the waste materials were allowed to dry in the open air under exposure to sunlight for about 24 h. Complete dehydration was accomplished in an electric oven at 105

°C for 4 h. Upon cooling to room temperature, the dried waste materials underwent sieving to obtain more uniform particle sizes and homogeneity to achieve powders in the micron range of these materials as a precursor to experimental investigation. All prepared waste-derived materials were stored in sealed containers to prevent any humidity.

For the preparation of the eggshell waste material, it was necessary to perform extensive washing of the collected eggshells with distilled water to remove all traces of organic matter present on the surface of the materials. Drying in an electric oven was performed at 200°C for 1 hour to achieve powder without humidity that could be used for manufacturing composite specimens. The crushing process took place after drying by grinding in a pestle and mortar for 15 minutes.

All prepared waste powders were analyzed using EDS and SEM techniques to examine their chemical compositions and morphological features, respectively.

2.2.2. Mortar Specimens Preparation

Small cylindrical specimens with an approximate diameter of 1 cm and a thickness of 3 mm were used for the antibacterial (biological) evaluation (as shown in Figure 1). The specimens included a control sample (cement, sand without any additives) and modified samples incorporating waste materials (copper-based waste, zinc oxide (ZnO), and eggshell powder). In these specimens, the waste materials were incorporated as additives at predetermined percentages to evaluate their effectiveness against bacterial growth within the cement matrix.

All specimens were prepared at room temperature and normal laboratory humidity conditions to ensure suitable consistency. After pouring, the samples were cured under identical conditions until complete hardening was achieved before antibacterial test.



Fig(1)mortar specimen

2.2.3- Epoxy Specimens

The epoxy specimens were fabricated by mixing resin and hardener at a volumetric ratio of 1:1, according to the manufacturer's guidelines. An equivalent percentage of additives was added to each fabricated epoxy specimen in order to determine the efficacy of the chosen waste-derived additives. A reference specimen with pure epoxy and without any additives was also manufactured as an indicator for comparing the performance of the fabricated composite specimens.

Disc-shaped epoxy samples having a diameter with thickness 5mm, 1mm (as shown in Figure 2). Full curing took place after 24 hours.



Fig (2) Epoxy specimen

2.3 Characterization of Waste Materials

To understand the microstructure and surface features of the tested waste-derived materials, EDS and SEM analyses were conducted. Elemental composition determination of the three investigated additives—eggshell powder (ESP), copper particles (CuO), and zinc oxide particles (ZnO)—was carried out via energy dispersive spectroscopy (EDS) technique. EDS tests were performed using a scanning electron microscopy instrument with an accelerating voltage of 30 kV. Sample selection from each investigated type of additive was performed to obtain accurate elemental composition measurements. The obtained results serve as a basis for understanding antibacterial efficiency of each tested formulation. EDS analysis was performed to analyze the elemental compositions of the waste materials under investigation. Moreover, SEM evaluates surface textures and particle shapes. EDS analysis allowed for identifying the main chemical components of the tested waste materials and helped assess the purity level of these materials. The purity determines by the presence of any possible contaminants or other minor elements that could be detected by EDS analysis. The obtained data on elemental compositions are very important since they will provide valuable information for evaluating the applicability of these waste materials as functional construction additives. the surface microstructure of the produced waste-derived materials was analyzed via Scanning Electron Microscopy (SEM). SEM tests were carried out through the use of an Axia Chemi SEM device (Thermo Scientific) operating at 30 kV accelerating voltage

2.5 Physical tests

Physical test is important support the bacterial tests. Where the incorporation of waste material has decreased the voids inside the material leading to a reduction of the colonization of bacteria thus leading to less airborne bacteria (staph). This reduction accompanied by disabling the still micro paths in the material's void, directly reduces the water absorption and that stops the water transmitted bacteria (E. Coli.) from entering the inner structure of the material.

2.5.1 Density

Density analysis was carried out to evaluate the degree of compaction and microstructure characteristics of the synthesized cementitious and epoxy composites specimens containing waste additives. Density determination involved measurement of mass and volume parameters of the hardened composite specimens. Density was calculated according to equation (1)[13] :

$$\text{Density} = \frac{m}{v} \dots\dots\dots(1)$$

Density: g\cm³

m : mass (g)

v : volume (cm³)

2.5.2 Water Absorption

The ability of the developed cementitious and epoxy composites specimens to absorb water has been evaluated to evaluate the effect of incorporating waste-derived additives on the permeability properties and porosity structure of the composite samples. This test method provides an important parameter for evaluating resistance to moisture ingress that may transfer bacteria. Water absorption determination was conducted following the standards by the American Society for Testing Materials ASTM C642-97, ASTM D570-22. Before immersion, the specimens were oven-dried to obtain their dry weights. Then the specimens were immersed in distilled water for 24 hours in laboratory-controlled conditions. Upon immersion, any surface moisture content was thoroughly wiped out using a dry cloth to obtain the saturated weights. Water absorption for mortar was calculated according to equation (2)[13] :

$$\text{Absorption after immersion} = [(B - A)/A] \times 100 \dots\dots\dots(2)$$

Where;

A: mass of oven-dried specimen in air, g

B: mass of dry specimen in air after immersion, g

Water absorption for epoxy specimens was calculated according to equation(3)[14]:

$$\text{Water Absorption}\% = \frac{W_{wet}-W_{dry}}{W_{dry}} \times 100 \dots\dots\dots(3)$$

Where;

W_{wet}: The weight of specimen after immersion

W_{dry}: The weight of specimen before immersion

2.5.3 Porosity

Porosity analysis, according to ASTM C642-97[13], was conducted to determine the impact of waste-derived additives on the internal microstructure of the developed cementitious composites and epoxy specimens. Porosity is an important indicator that has a direct impact on water penetration and bacterial infiltration in cementitious materials and affects the stability of such constructions over time. Lower porosity leads to increased density of the formed cement matrix and limits moisture migration, thus preventing the establishment of microorganisms. For epoxy composites specimens, the porosity affects the quality of the antibacterial coating.

The porosity measurements were performed based on weight changes after complete submersion of the tested specimens in water. The acquired data were subsequently used for determining the percentage value of porous cavities inside the composite sample. Porosity was calculated according to equation (4)[13] :

$$\text{porosity \%} = \frac{W_2 - W_1}{W_2 - W_3} \times 100 \dots\dots(4)$$

Where:

W1: the average dry weight of the specimen, g

W2: the average wet weight of the specimen,g

W3: the average immersed weight of the specimen,g

2.6 Antibacterial Tests

2.6.1 Antibacterial Activity Test (Agar Well Diffusion Method)

The antibacterial activity test was carried out using the agar well diffusion method to compare between waste powders and pure materials, beside the test was accomplished to know the antibacterial activity of epoxy- waste composites. Approximately 20 mL of Mueller–Hinton agar was poured into sterile Petri dishes and allowed to solidify under sterile conditions. The selected bacterial strains, *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative), were inoculated uniformly on the agar surface using a sterile loop [12], [15], [16].

After inoculation, wells of 6 mm diameter were prepared in the agar using a sterile tip. The prepared samples were introduced into the wells at different concentrations.

The Petri dishes were then incubated at 37°C for 24 hours. After the incubation period, the diameter of the clear inhibition zones formed around each well was measured in millimeters (mm) to determine the antibacterial activity of the tested samples[17], [18].

2.6.2 The Colony Forming Unit (CFU)

The antibacterial activity of the cementitious samples was evaluated using the Colony Forming Unit (CFU) method to assess the effectiveness of waste-derived materials as additives within the cement matrix.

First, the culture media were prepared under sterile conditions. Nutrient agar was prepared using 350 mL of distilled water, which was sufficient for approximately 17 Petri dishes for each bacterial type. In addition, nutrient broth was prepared by dissolving 4.55 g of nutrient broth powder in 350 mL of distilled water, followed by sterilization. After sterilization, 10 mL of the prepared nutrient broth was transferred into each sterile vial.

For each treatment, four replicates were prepared. A volume of 100 µL of a 24-hour-old bacterial culture was added to each vial containing 10 mL of nutrient broth. Then, 1 g of the cementitious sample containing waste-derived additives was introduced into each vial. The prepared vials were incubated in a shaking incubator at 37°C for 24 hours.

After the incubation period, 5 mL of sterile distilled water was added to each vial and mixed thoroughly. Then, 10 µL of the resulting suspension was taken and inoculated onto nutrient agar plates using the swabbing method. The inoculated plates were incubated in a standard incubator at 37°C for another 24 hours, as shown in Figure 3 [19].

Finally, the number of bacterial colonies formed on each plate was counted, and the results were used to evaluate the antibacterial efficiency of the waste-derived materials when incorporated into cementitious composites.

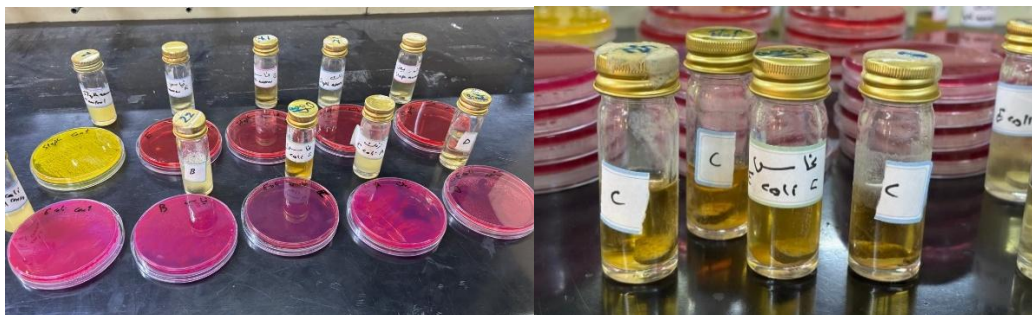


Fig.(3) Specimen of the Colony Forming Unit test

3 Results and Discussion

3.1 Characterization of Waste Materials

3.1.1 EDS Analysis of Eggshell Powder (ESP)

The EDS analysis of calcined eggshell powder (ESP) highlights its elemental nature, consisting mainly of calcium (Ca), oxygen (O), and carbon (C). As in Table 8 and with their spectra depicted in Figure 4, the analysis produced a total count of 46,324 counts and an average count rate of 769 counts per second (cps) in a measurement period of 54 seconds. Elemental weight fractions of 38.9 wt% of Ca, 49.3 wt% of O, and 11.9 wt% of C have been calculated, representing Ca = 19.3 at%, O = 61.1 at%, and C = 19.6 at%. The data are consistent with the predicted elemental stoichiometry of calcium carbonate (CaCO_3), representing approximately 94-97 wt% of eggshell. The intensity peak of Ca $K\alpha$ indicates high purity of the

investigated material. Carbon and oxygen are attributed to the presence of calcium carbonate. The presence of negligible amounts of contamination peaks suggests the successful removal of contaminants during the pre-treatment washing and drying process performed on eggshell. The high calcium fraction in ESP confirms the material's applicability as a supplementary cementitious additive or as an additive in composite materials.

Table (8). EDS elemental composition of Eggshell Powder (ESP)

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
C	19.6	± 0.6	11.9	± 0.3
O	61.1	± 1.4	49.3	± 1.2
Ca	19.3	± 0.2	38.9	± 0.4

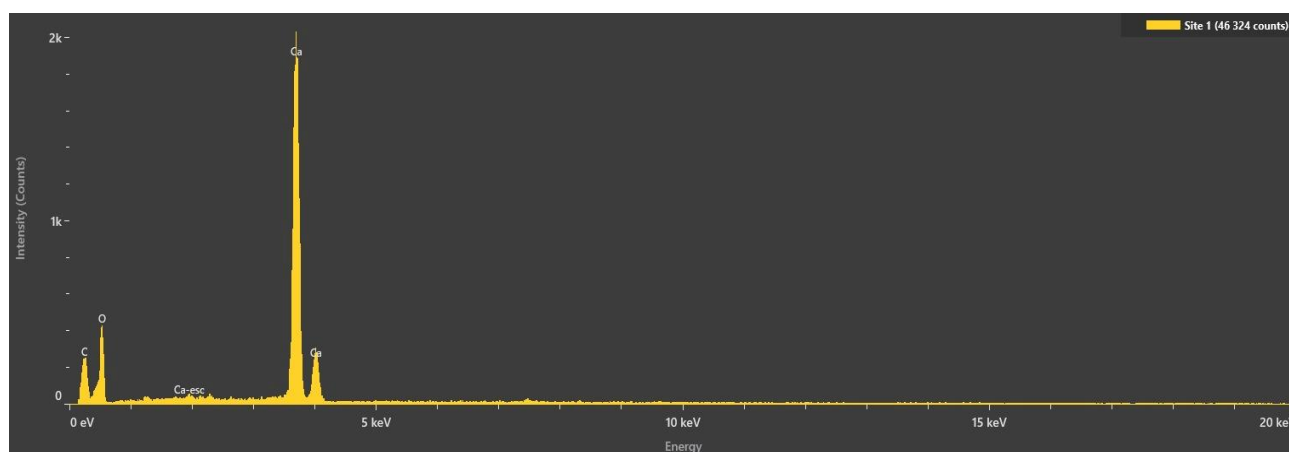


Fig 4. EDS spectrum of Eggshell Powder (ESP)

3.1.2 EDS Analysis of Copper particles (Cu)

Energy dispersive spectroscopy (EDS) analysis of the copper (Cu) particles indicated a total particle count of 57,410 at a mean count rate of 441 counts per second (cps). The EDS analysis entailed two spectral investigations: a full-site investigation, which involved the identification of all detected elemental components, and a selective Cu element investigation. The analytical findings obtained through both approaches are summarized in Tables 9 and 10, respectively, with their spectra depicted in Figures 5 and 6. According to the full elemental spectrum (Table 9), Cu was identified as the predominant metal component, constituting 51.0 wt% (19.4 atomic percent (at%)). Other detected elements include C (28.6 wt%, 57.5 at%), O (12.0 wt%, 18.1 at%), Si (2.2 wt%, 1.9 at%), Ca (2.6 wt%, 1.6 at%), and Fe (3.6 wt%, 1.5 at%). Trace elements such as Si, Ca, and Fe were detected and attributed to possible contamination during sampling or analysis,

and not related to the investigated Cu particles. Selective analysis of the Cu particles revealed a Cu–O binary composition with a weight percentage of 84.2 wt% (atomic percentage of 57.3 at%) and 15.8 wt% (42.7 at%), respectively. These values were nearly identical to those expected from stoichiometric CuO, which consists of Cu (79.9 wt%) and O (20.1 wt%). Furthermore, typical emission peaks of Cu, including Cu L α and Cu K α peaks, were identified, with a prominent Cu K α peak near 8 keV. Confirmatory evidence suggests the presence of relatively pure (i.e. with minimum impurities) Cu particles.

Table (9). EDS full elemental composition of Cu particles

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
C	57.5	± 1.3	28.6	± 0.6
O	18.1	± 0.9	12.0	± 0.6
Si	1.9	± 0.1	2.2	± 0.1
Ca	1.6	± 0.0	2.6	± 0.1
Fe	1.5	± 0.1	3.6	± 0.2
Cu	19.4	± 0.3	51.0	± 0.7

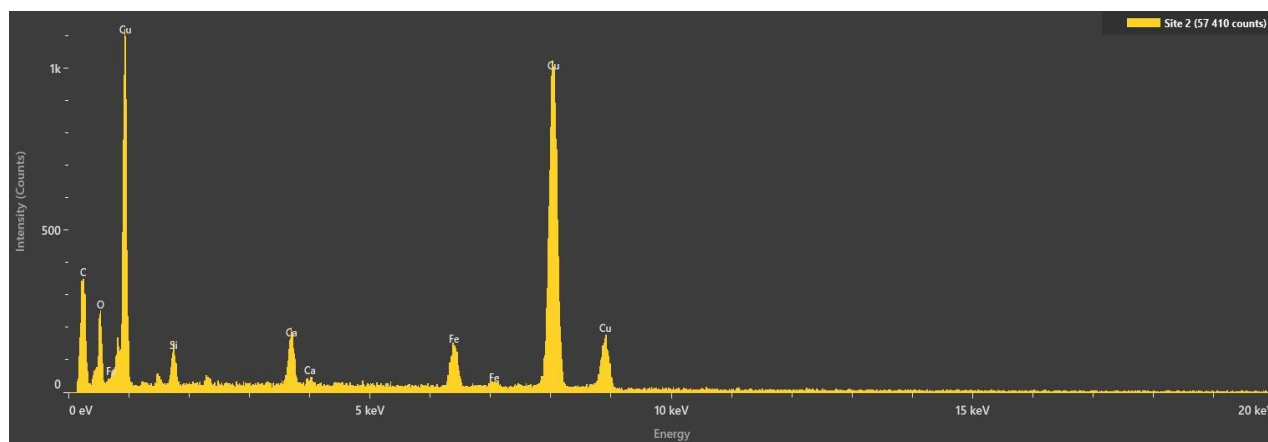


Fig. (5). EDS spectrum of CuO particles — Full elemental spectrum

Table (10). EDS refined Cu phase composition (Cu and O only)

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
O	42.7	± 1.5	15.8	± 0.5
Cu	57.3	± 0.8	84.2	± 1.1

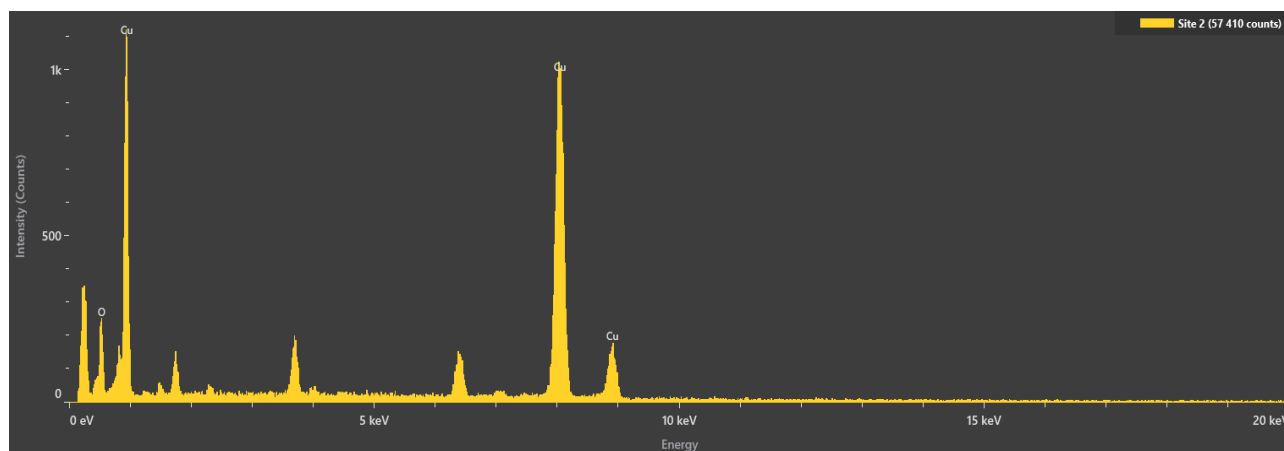


Fig. (6). EDS Spectrum of Cu Particles — Refined Cu–O Spectrum.

3.1.3 EDS Analysis of Zinc Oxide Particles (ZnO)

Elemental analysis performed on a zinc oxide particle obtained from waste materials using the EDS technique revealed 249,730 counts collected during a data acquisition time period of 97 seconds, yielding an average count rate of 2,769 cps. Two elemental analyses were carried out: a general full-site elemental analysis and a focused analysis for zinc-oxygen pairs. Data were summarized in Tables 11 & 12, while Figures 7 & 8 depict the obtained EDS spectra. Full-site elemental analysis resulted in zinc being the primary constituent, accounting for 91.0 wt% (73.4 at%), whereas oxygen was determined to account for 7.7 wt% (25.4 at%). A small amount of nickel was also detected at concentrations of 1.3 wt% (1.2 at%). Trace amounts of nickel represent the emission contribution of nickel-coated stubs commonly used during sample preparation for the SEM-EDS technique rather than impurities within zinc oxide particle compositions. Zinc-oxygen elemental analysis revealed a two-element zinc-oxygen compound with 92.1 wt% (73.9 at%) zinc and 7.9 wt% (26.1 at%) oxygen contents, confirming the purity of the tested zinc oxide particles upon the removal of signals emitted by substrates. The EDS spectrum is characterized by sharp zinc lines, including prominent zinc $L\alpha$ (~1 keV) and zinc $K\alpha$ (~8.6 keV) emission peaks. An exceptionally high signal-to-noise ratio, together with relatively large counts, confirms a highly crystalline nature of the tested ZnO particles. These findings support the purity of zinc oxide applied throughout the experiments.

Table (11). EDS full elemental composition of ZnO particles

Element	Atomic %	Atomic % Error (±)	Weight %	Weight % Error (±)
O	25.4	± 0.5	7.7	± 0.1
Ni	1.2	± 0.1	1.3	± 0.1
Zn	73.4	± 0.5	91.0	± 0.6

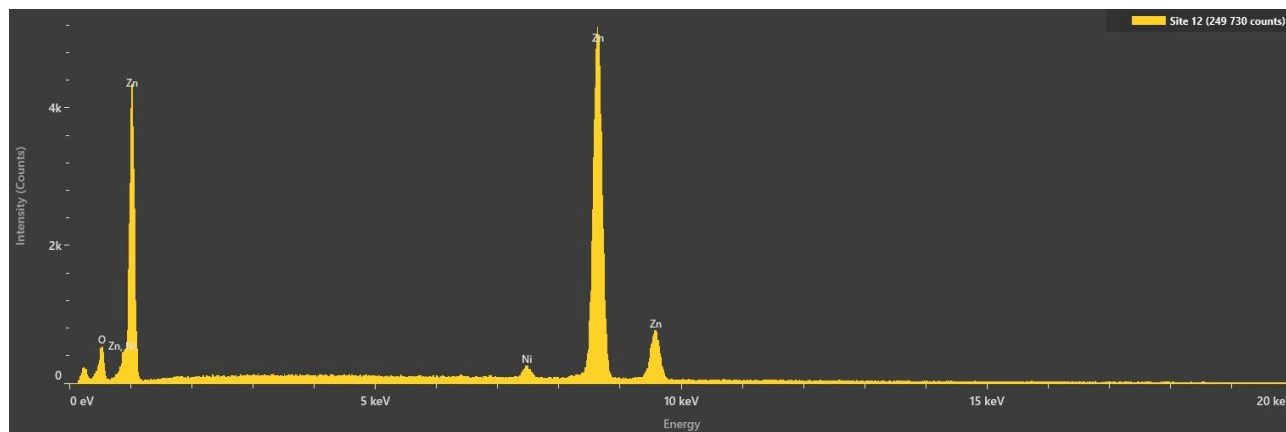


Fig. (7). EDS Spectrum of ZnO particles — Full Elemental Spectrum

Table (12). EDS Refined ZnO Phase Composition (Zn and O only)

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
O	26.1	± 0.5	7.9	± 0.2
Zn	73.9	± 0.5	92.1	± 0.6

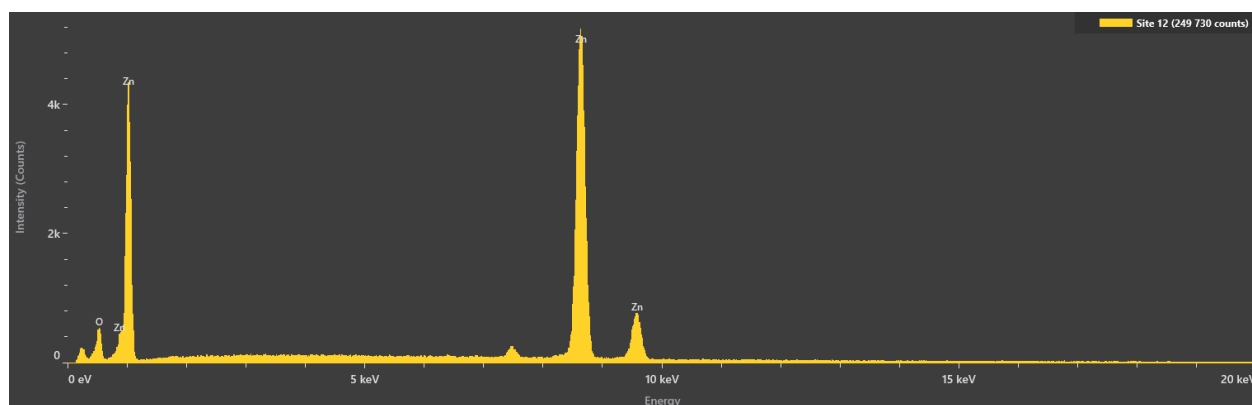


Fig (8). EDS spectrum of ZnO particles — Refined Zn–O Spectrum

3.1.4 SEM Morphological Analysis of Waste-Derived Powders

Microstructural images of waste-derived eggshell powder (ESP), copper oxide (CuO), and zinc oxide (ZnO) are shown in Figures 9-10-11, respectively. Microstructural analyses demonstrate that each investigated additive exhibits distinctive characteristics associated with specific functionality. Therefore, the present work seeks to elucidate the relationships between microstructural attributes and functional performance.

As illustrated in Figure 9, the investigated ESP micrograph acquired at 2000x magnification shows densely packed, irregular platelike and lamellar calcium carbonate CaCO_3 calcite polymorph structures. Irregular crystals possess mostly planar and leaflike morphology accompanied by sharp and angular edges. Particle size exhibits high variation owing to the mechanical grinding and sieving processes used throughout material synthesis. Individual crystals display fracture faces indicative of brittle cleavage induced by mechanical processing. Small submicron particles dispersed in large crystals could promote enhanced densification when added to cementitious media or other media. From the perspective of the proposed densification mechanism, such a microstructural configuration ensures favorable filler effects and densification promotion.

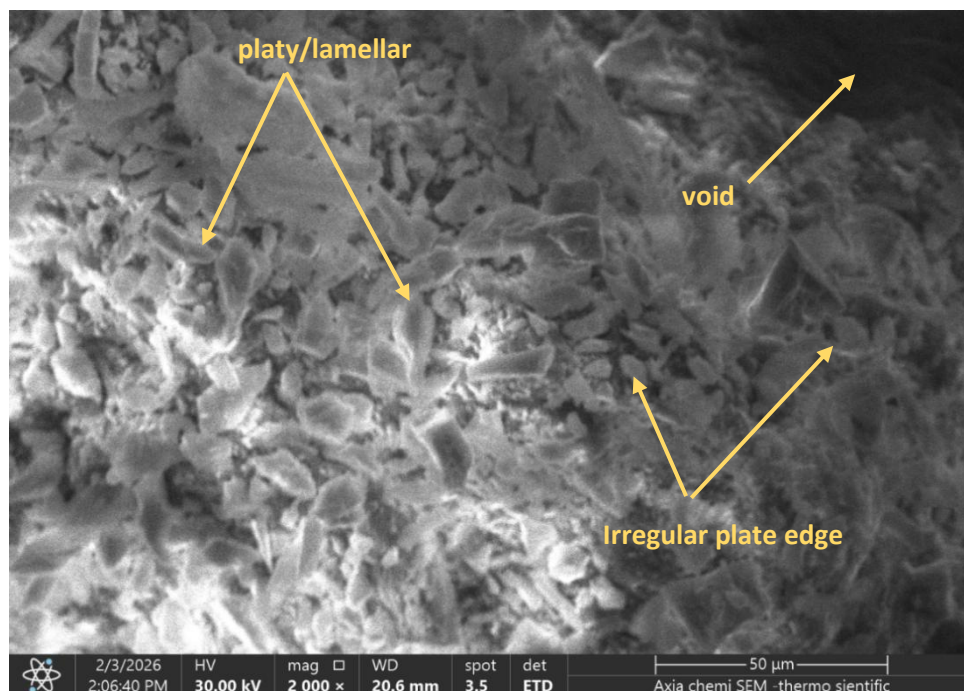


Fig. (9). SEM of ESP.

On the contrary, microstructural features observed for CuO particles illustrated in Figure 10 exhibit distinct differences compared to ESP. Cu particles show heterogeneous crystal shapes characterized by loose aggregates, significant presence of pores and voids in-between crystallites, as well as highly corrugated and rough surfaces. Large variations were identified concerning particle size and irregularity of crystal boundaries due to the natural formation process in an industrial environment. Such microstructure ensures a large crystal surface contributing to excellent bactericidal properties of copper-based compounds. On the mechanistic side, high surface area allows increasing the interfacial contacts of copper with aqueous media, enhancing the dissolution rate of Cu^{2+} ions and promoting their direct membrane-disruption action on pathogens. Hence, it is possible to state that copper microstructural characteristics directly affect its antibacterial activity, confirming theoretical considerations on the importance of the crystal surface.

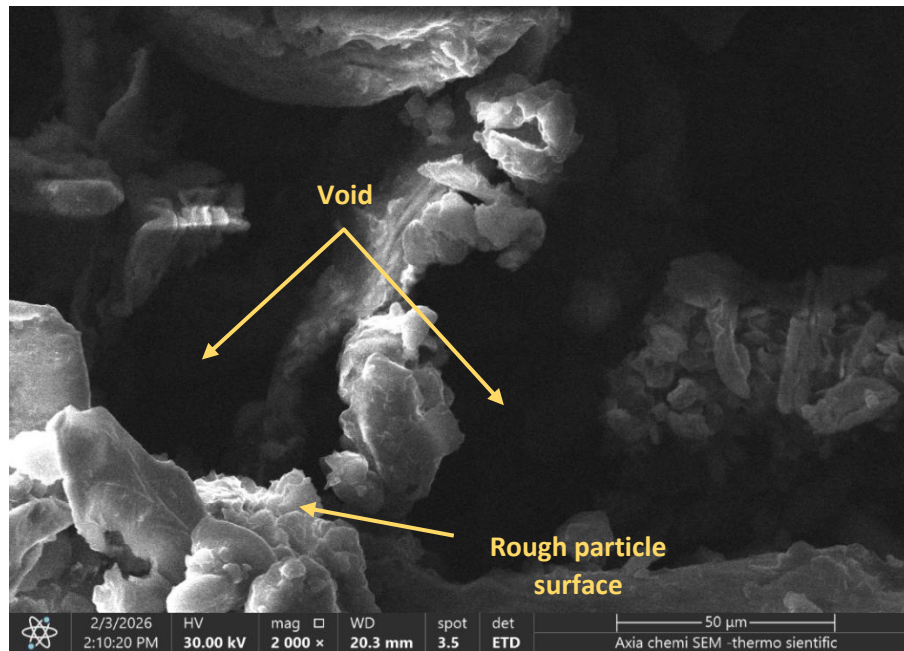


Fig. (10). SEM of Cu Powder.

Lastly, micrographs acquired for ZnO (Figure 11) exhibit distinct features as well. Micrographs obtained at 4000x magnification revealed the aggregation of ZnO particles into spherical clusters composed of uniformly sized submicron grains. Efficient agglomeration ensures a sufficiently high surface area of materials important for antibacterial activities. Nevertheless, micrographs obtained for ZnO clearly demonstrate an inclination towards agglomeration of materials, explaining the retardation phenomena observed in ZnO-replacement cementitious mortars.

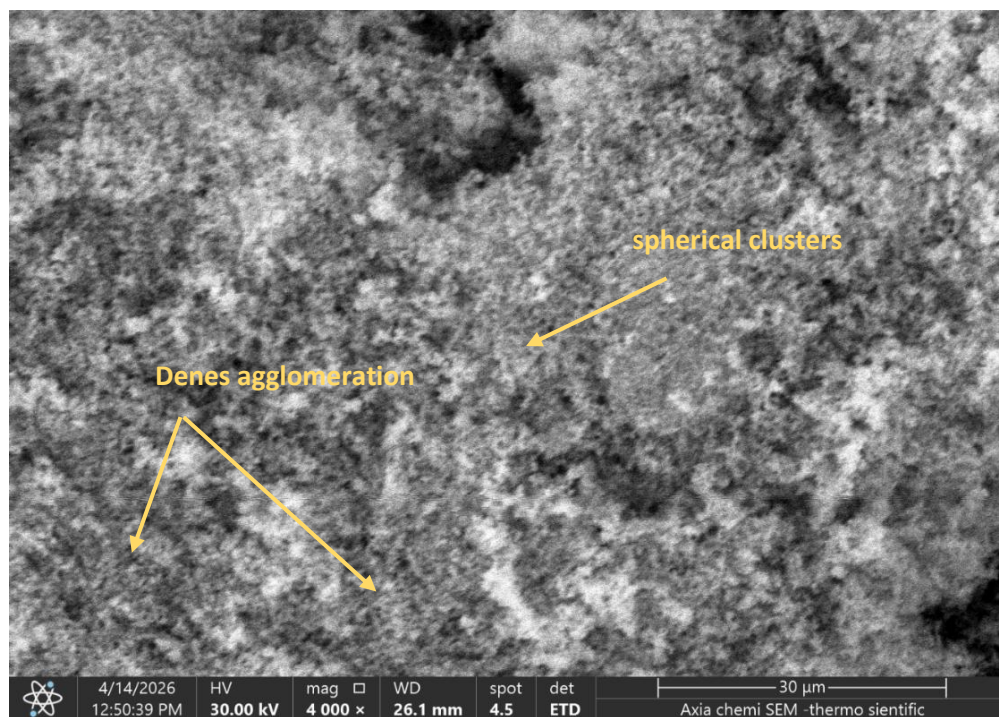


Fig. (11). SEM of ZnO Powder.

3.2 Physical Tests

3.2.1 Epoxy Specimens

According to the results of the physical tests presented in Table (13), using epoxy in surface coating shows great improvement in the physical properties of the samples. The result presents nil porosity and water absorption. The coating is characterized by its structure, which is thick and cohesive in nature, thus preventing water absorption into the internal structure of the sample. Because moisture and porosity play a vital role in colonization and growth of microorganisms, their absence greatly diminishes the risk of the surface being contaminated with bacteria.

Table(13) Physical tests of Epoxy

Waste materials	Density g\cm ³	Porosity %	Water Absorption %
CuO	5.3	Nil	Nil
ZnO	5.1	Nil	Nil
ESP	4.8	Nil	Nil

3.2.2 Cementitious Specimens

3.2.2.1 Density

Water absorption and porosity are inversely proportional to density; thus, a low density indicates high water absorption and porosity and possible bacterial establishment and transfer in porous material during water absorption. That is the reason why density testing is significant in determining antibacterial activity.

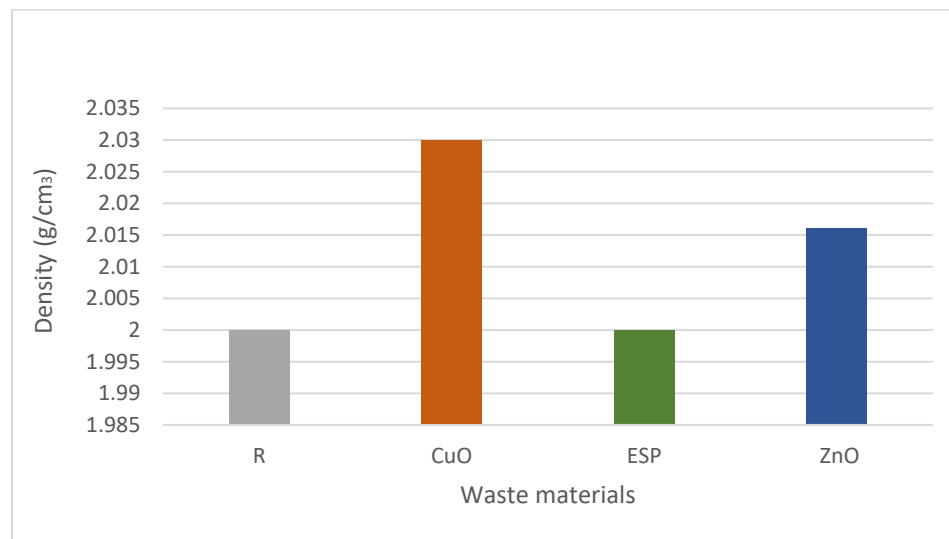


Fig. 12 Effect of waste materials on density

Density measurements of the hardened cementitious composites after 28 days are presented in Figure 12.

The reference specimen was used as a baseline for comparison with the remaining results after the addition of waste materials. The specimen containing copper waste showed a higher density, indicating that the copper particles contributed to improving particle packing within the cement matrix. This is due to the filler effect of copper powders, which reduces porosity and thus inhibits bacterial growth. In contrast, the zinc oxide waste specimen showed a slight rise compared to the reference specimen, attributed to the formation of calcium zincate $[\text{Ca}(\text{Zn}(\text{OH})_3)_2 \cdot 2\text{H}_2\text{O}]$, which features a lower density compared to the main hydration product C–S–H gel [20] [21].

Conversely, ESP-modified composites exhibited reduced density values. Such phenomena coincide with the relatively smaller specific gravity of eggshells in comparison with ordinary Portland cement clinker. Nevertheless, despite such a reduction, it should be noted that this decrease did not impair the matrix structure since the microfiller properties of CaCO_3 particles improved the density due to filling of micro-pores within the cementitious composite structure [22].

3.2.2 Porosity

A strong positive relationship between permeable porosity and water absorption values was observed across all mix series. Microstructural changes caused by the introduction of waste-derived materials have a pronounced impact on the permeability of the investigated cementitious systems. Antibacterially, this porosity change is important since it limits fluid (liquid or gas) pathways responsible for providing nutrients for microorganisms and minimizes available inner surfaces needed for biofilm attachment – processes complementing ion-mediated antibacterial properties [23].

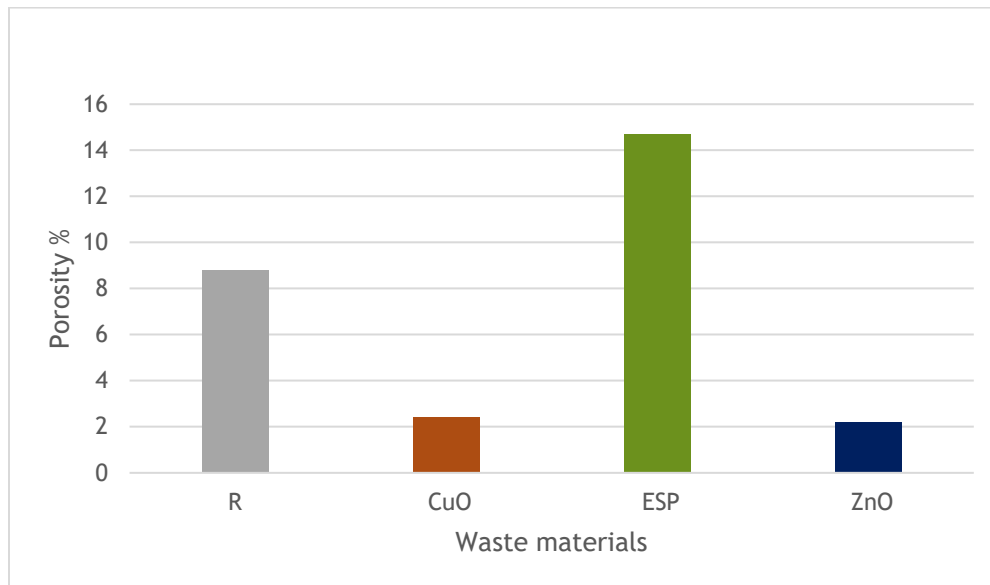


Fig.13 Effect of waste materials on porosity

Results of permeable porosity measurements are reported in % of total specimen volume in Figure 13. After comparison with the reference specimen, the following was found. Addition of copper oxide (CuO) resulted in a notable reduction in porosity value. Such a significant reduction in porosity is attributable to the ability of ultra-fine copper oxide micro-particles to function as efficient micro-filler

particles and prevent capillary connectivity and dense bonding within paste-aggregate interface zones. Cementitious composites containing ZnO modifier showed a similar tendency towards porosity reduction, which is slightly lower compared to values obtained with copper-based cementitious composites. Such a tendency could be explained by the additional ability of ZnO particles to fill existing pores regardless of hydration retardation effects due to zincate complex formation [20].

Specimens containing ESP additive demonstrated higher porosity percentages compared to reference values across all investigated substitution levels. This finding could be explained by two main factors: partial substitution of highly reactive clinker components for less active ones, resulting in decreased amounts of C-S-H gels formed during hardening; and relatively large particle size distribution of eggshell particles compared to ordinary Portland cement.

3.2.3 Water Absorption

Water absorptivity relates negatively to the aggression of certain ions, such as sulfate, chloride, and carbonation reactions. Such factors contribute significantly to accelerated deterioration rates and the creation of an environment favorable for Microbially Induced Concrete Corrosion (MICC) [23]. Thus, the performance of water absorption resistance in copper-oxide and zinc oxide-containing mixes highlights antimicrobial benefits provided by the respective waste materials.

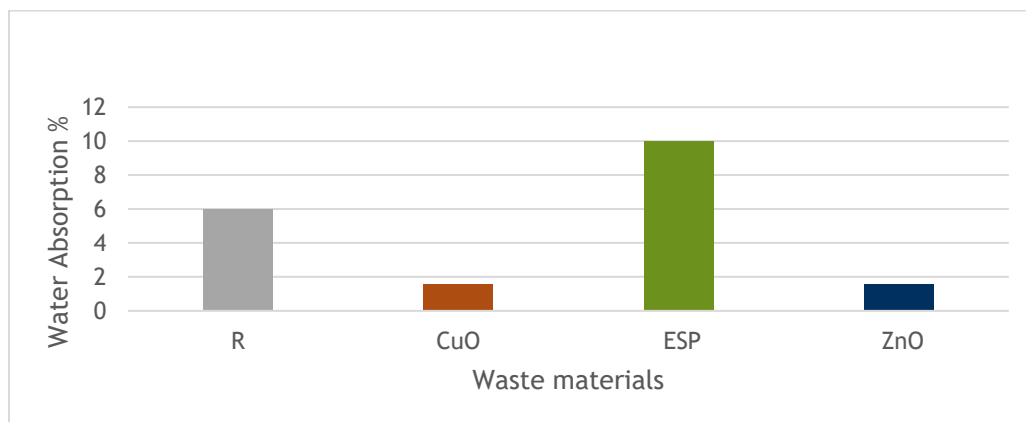


Fig.14 Effect of waste materials on water absorption

The values of water absorption capacity (%), the percentage increase in mass after soaking for 24 hours, are listed in Figure 14. After comparison with the reference specimen. Specimens with copper modification exhibited a consistently decreasing trend with regard to water absorption. Such observations correlate well with porosity trends due to the filler effects of finely dispersed copper particles preventing capillary action and decreasing the interconnectivity of pores. Consequently, such changes significantly impede the process of moisture infiltration in the cementitious system.

Zinc oxide-modified specimens also exhibited the same trend concerning water absorption capacity. These observations indicate that the blocking effect caused by the formation of finer pores as a result of increased ZnO particle concentration outweighs any negative impacts on pore network disruption resulting from the precipitation of zinc-containing compounds.

The specimens comprising eggshell powder (ESP) recorded the highest water absorption compared to the rest of the tested specimens and exceeded the control specimen.

3.3 Antibacterial Activity Test (Agar Well Diffusion Method)

Analysis of waste-derived material performance relative to their pure counterparts provides valuable information regarding the viability of cost-effective sustainable substitutes. The current evaluation seeks to prove the potential of using waste materials as antibacterial functional additives, ensuring sustainability and material savings.

3.3.1 Powders

Antibacterial efficacy testing was performed on CuO pure, CuO waste, ZnO pure, ZnO waste, CaCO₃ pure, and ESP waste against *S. aureus* and *E. coli* by means of the Agar Well Diffusion technique. According to Table 14, all materials demonstrate significant antibacterial activity against *S. aureus* and *E. coli*.

Copper-containing materials (waste CuO and pure CuO) exhibit notable antibacterial properties due to the release of Cu²⁺ ions, which react with bacterial cell walls and membranes to increase their permeability, disturb fundamental physiological activities, and lead to the leakage of intracellular compounds. Additionally, copper ions have been shown to promote oxidation and generate free

radicals (reactive oxygen species) responsible for inhibiting bacteria. As shown in Fig. (15) and (16).

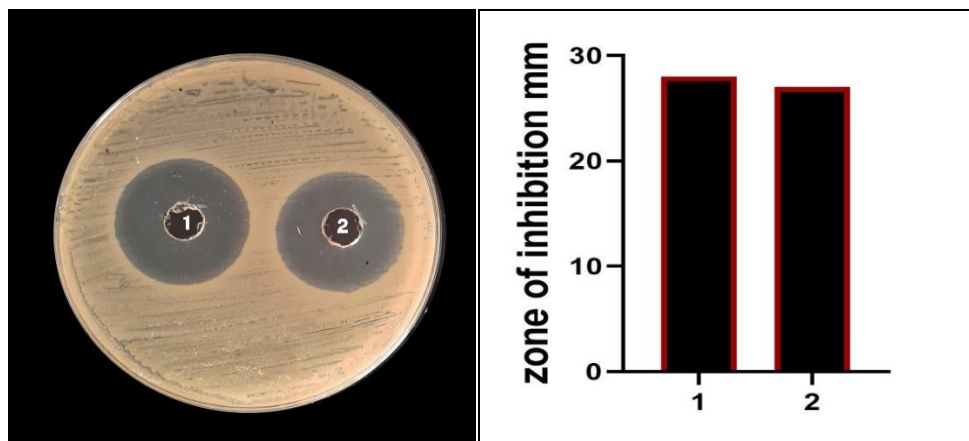


Fig. (15). Antibacterial activity of (Test material) against *S. aureus*. 1, (pure CuO). 2, (Copper oxide waste).

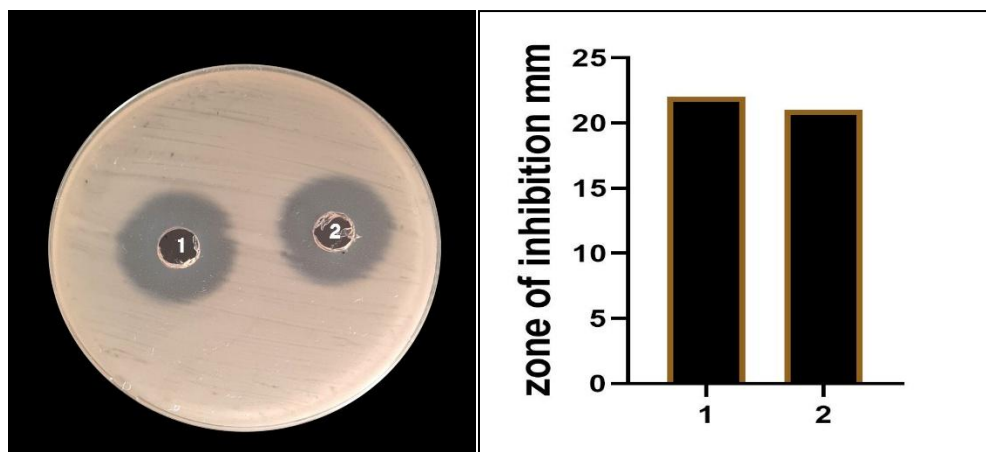


Fig. (16). Antibacterial activity of (Test material) against *E. coli*. 1, (pure CuO). 2, (Copper oxide waste).

Pure ZnO and ZnO waste demonstrate prominent antibacterial efficacy. Their antibacterial mechanism involves generating free radicals (reactive oxygen species) and releasing Zn^{2+} ions, which oxidize cellular structures such as proteins, lipids, and DNA molecules. The higher efficacy observed for ZnO waste compared to pure ZnO might result from changes in the material's microstructure (particle morphology, surface area), which facilitates improved ion release rates and increased generation of reactive oxygen species. In particular, such microstructural properties contribute to accelerated dissolution of ZnO particles with the release of

Zn^{2+} cations and subsequent formation of ROS species. Both actions account for crucial bactericidal effects. As shown in Fig. (17) and (18).

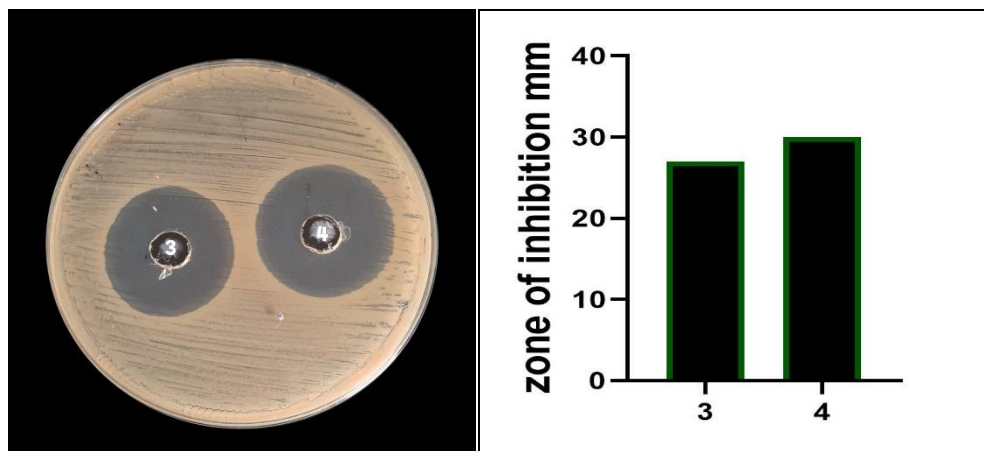


Fig. (17) Antibacterial activity of (Test material) against *S. aureus*. 3, (pure Zinc oxide). 4, (ZnO waste).

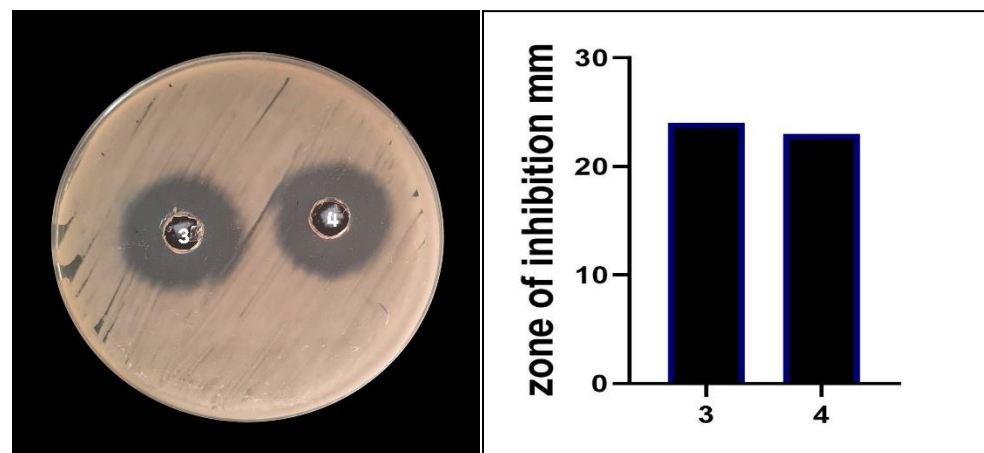


Fig. (18) Antibacterial activity of (Test material) against *E. coli*. 3, (pure Zinc oxide). 4, (ZnO waste).

Calcium carbonate (pure CaCO_3 and ESP) exhibits considerable antibacterial activity against the chosen bacteria. Relatively large inhibition zones indicate that their bactericidal action may occur through altering the pH value at the contact interface and gradual ion release (calcium ions), which disrupts bacterial physiology. As shown in Fig. (19) and (20).

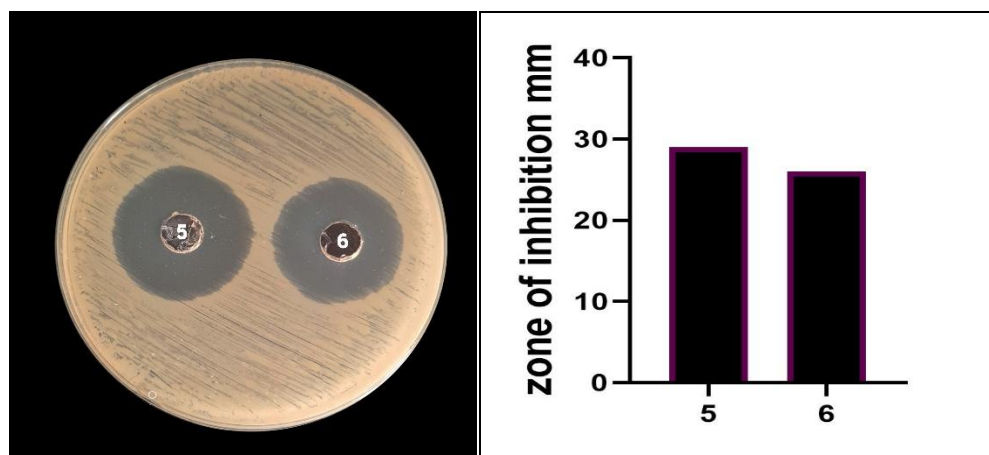


Fig. (19) Antibacterial activity of (Test material) against *S. aureus*. 5, (pure CaCO_3). 6, (waste Egg Shell).

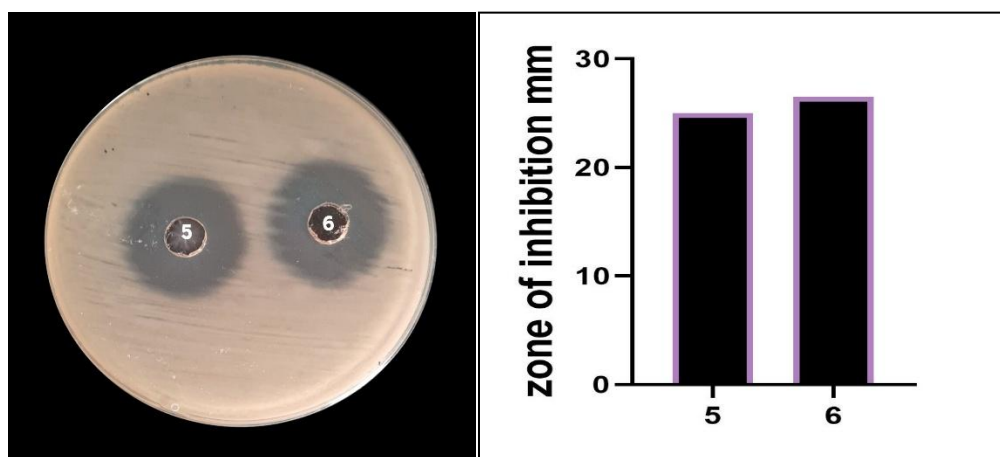


Fig. (20). Antibacterial activity of (Test material) against *E. coli*. 5, (pure CaCO_3). 6, (Egg Shell waste).

S. aureus is a Gram-positive bacterium, whereas *E. coli* is a Gram-negative bacterium. Results revealed that all tested materials were successful in generating inhibition zones for both Gram-positive and Gram-negative bacteria, with a higher killing of *S. aureus* toward the vast majority of investigated antibacterial materials. ZnO waste generated the greatest inhibition zone among all materials, followed closely by CaCO_3 , CuO, Cu waste, and pure ZnO, exhibiting excellent antibacterial performance against the aforementioned bacterial strain. Increased susceptibility may be associated with reduced complexity of bacterial cell walls and the absence of additional protective barriers, such as outer membranes, enabling metal ions and ROS entry inside bacterial cells and facilitating efficient bacterial elimination. *Escherichia coli* was found to display minimal sensitivity towards most of the tested materials. The reduced susceptibility may be attributed to greater bacterial complexity, including the presence of an extra layer of outer membrane, which

makes it difficult for antibacterial agents and metal ions to diffuse into bacterial cells. However, all tested materials were successful in generating inhibition zones for both Gram-positive and Gram-negative bacteria. This finding confirms their ability to inhibit bacterial growth and supports their utilization as antibacterial additives.

Table (14). Antibacterial analysis of CuO, ZnO, and EPS samples.

Antibacterial Analysis (Zone of Inhibition (mm))						
Sample	1 (CuO) pure	2(CuO Waste)	3 (ZnO) pure	4 (ZnO Waste)	5 (CaCO ₃) pure	6 (ESP)
<i>S.aureus</i>	28	27	27	30	29	26
<i>E.coli</i>	22	21	24	23	25	26.5

Amongst all materials, ESP generated the largest inhibition zone for *E. coli* bacteria, followed by pure CaCO₃ and pure ZnO. Lower antibacterial efficiency was observed in copper oxide and ZnO materials. ZnO waste had the largest inhibition zone for *S.aureus*, followed by CuO waste and ESP. This observation highlights the significance of bacterial cell wall structure in estimating the susceptibility towards metal-based antibacterial materials; results still highlight promising antibacterial efficacy and viability of using inexpensive waste-derived material. As shown in figure 20.

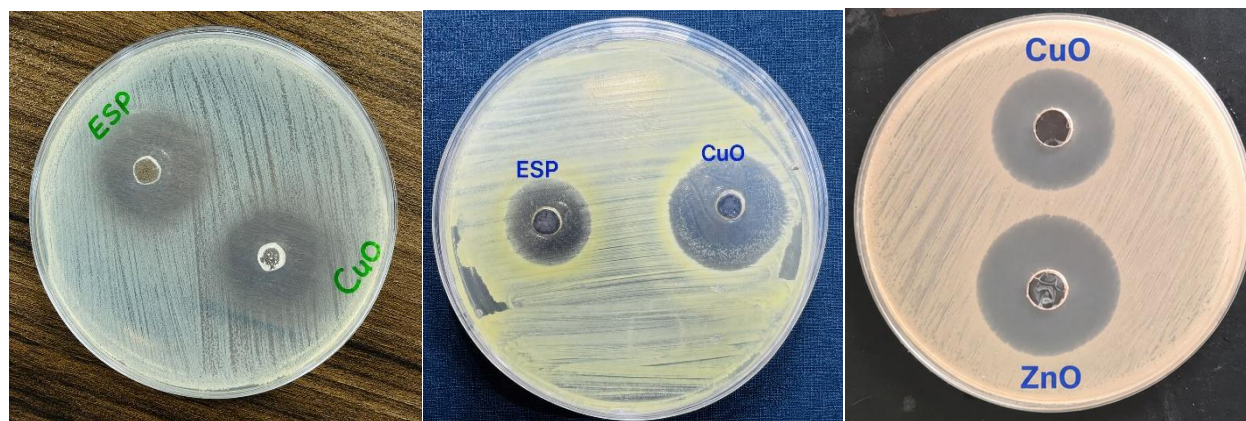


Fig.(20) a. The comparison between EPS and CuO for killing *S.aureus* **b.**The comparison between EPS and CuO for killing *E. coli* **c.**The comparison between ZnO and CuO for killing *E. coli*

3.3.2 Epoxy

For practical applications, epoxy has been assessed as a possible coating on substrate material.

Table 15 confirms that the control epoxy specimen did not display any antibacterial properties against either bacterial strain (inhibition zone = 0 mm).

Table (15). Antibacterial analysis for epoxy composite samples.

Antibacterial Analysis (Zone of Inhibition (mm))				
Sample	A(Control epoxy)	B(ZnO)	C(CuO)	D(ESP)
<i>E.coli</i>	8	16	18	8
<i>S.aureus</i>	0	19	22	19

This means that epoxy can't kill bacteria but could stop them from growing. The lack of reactivity stems from the absence of ion release and chemical inertness without any functional group capable of reacting with the bacterial cells. As shown in Fig. (21) and (22).

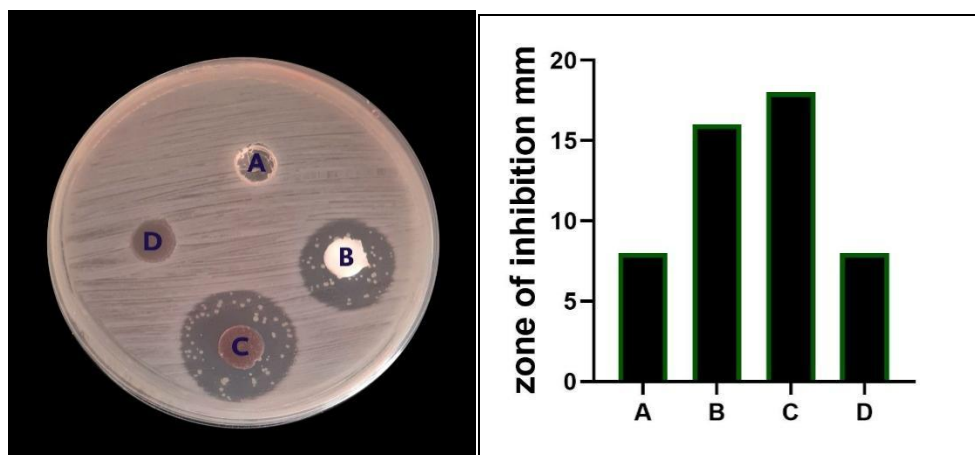


Fig. (21). Antibacterial activity of (Test material) against *E.coli*. A, (Control Epoxy). B, (Zinc waste). C (Copper waste). D, (Egg Shell).

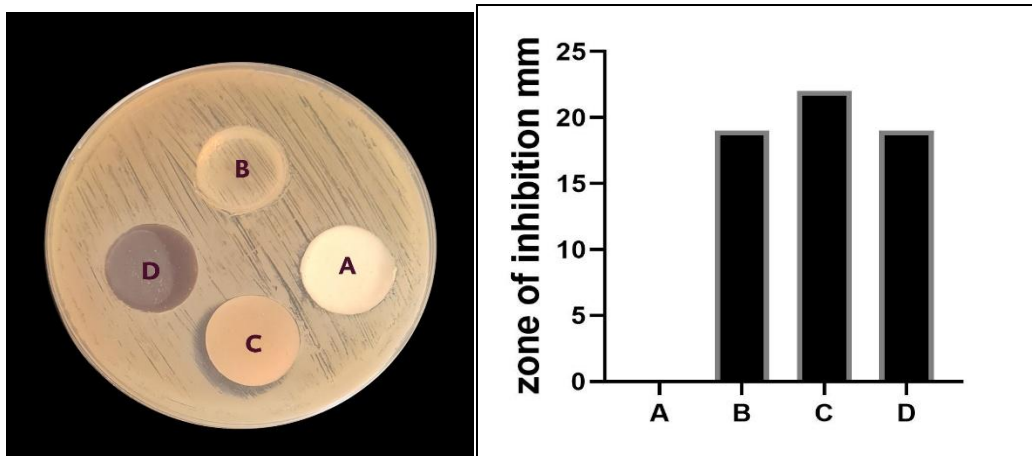


Fig. (22). Antibacterial activity of (Test material) against *S.aureus*. A, (Control Epoxy). B, (Zinc waste). C, (Copper waste). D, (Egg Shell).

Promote the release of free metal ions (Cu^{2+} and Zn^{2+}). These ions affect the integrity of bacterial cell walls by altering the functionality of the proteins and increasing their permeability, thereby causing leakage of intracellular content and subsequent death of bacterial cells [19], [24]. Additionally, metal ions stimulate the production of ROS, which cause oxidative stress in bacterial cells through attacks on the essential biological molecules, such as proteins, lipids, and DNA [25], [26]. Moreover, contact between the material surfaces and bacterial cells leads to membrane rupture and the inhibition of bacterial multiplication [27]. The studied epoxy coatings modified with eggshell powder (ESP) proved to have clear antibacterial activity through reducing the number of cultured microbes present in the setting. This is due to the fact that eggshell powder contains such a substance in its active form, which can break the viability of microbial cells by means of surface activity and the influence of the alkali environment. No matter how important this property of ESP is, copper oxide (CuO) still remains the most effective antibacterial agent comparing the previously tested additives since it shows the best antibacterial efficiency in relation to both types of bacteria: Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*) through the production of oxidative stress and $\text{Cu}^{2+}/\text{Cu}^{+}$ ions in the case of the additive based on copper.

3.4 The Colony Forming Unit (CFU)

Antibacterial activity of cement-based composite materials containing waste-derived antibacterial agents was quantitatively estimated through CFU counting. The findings revealed that the introduction of waste-derived antibacterial additives led to an increase in the antibacterial performance of cement-based composite materials as compared to the control (consisting of the bacterial suspension) and reference specimens (representing plain cement mortar without any antibacterial additive). It was found that antibacterial efficacy strongly depended on both the type of incorporated additive material and the strain of bacteria tested.

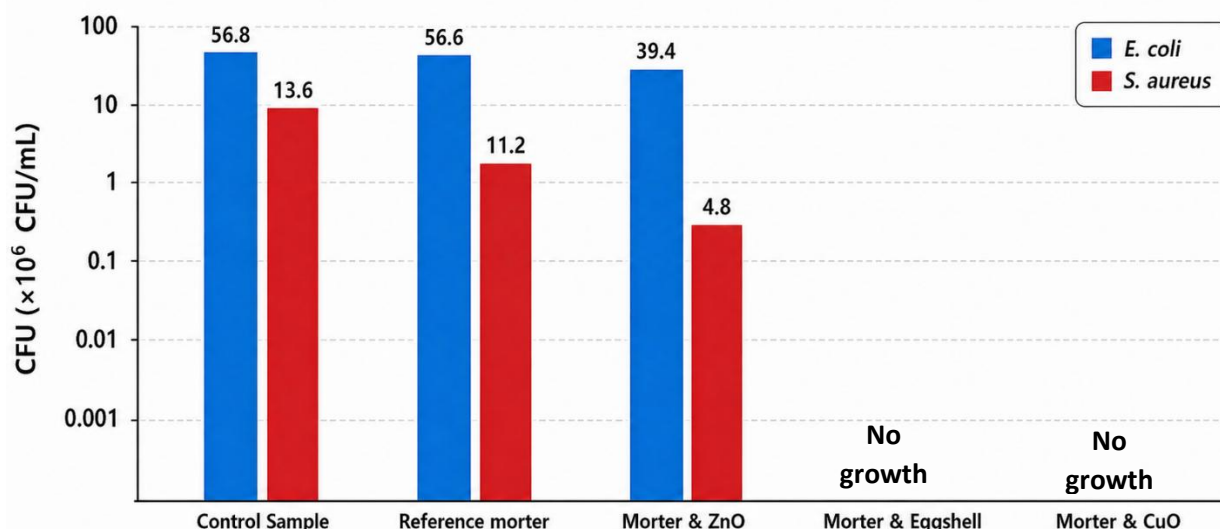


Fig. (23). Comparative Antibacterial Activity of Modified Mortar Samples Against *E. coli* and *S. aureus*

Significant bacterial growth in control and reference specimens confirmed poor intrinsic antibacterial capability in pure cementitious matrices. On the other hand, cementitious matrices modified by zinc oxide displayed evident reductions in bacterial colony counts for both types of bacteria, as shown in Figure 23. Hence, this finding corroborates the antibacterial efficacy of zinc oxide against the two investigated strains. Furthermore, specimens containing eggshell powder displayed notable inhibition in bacterial growth, which denotes remarkable antibacterial efficacy within the cementitious matrix.

According to Table 15-16, the results demonstrate the superior antibacterial effectiveness achieved through the incorporation of copper in cementitious samples by effectively inhibiting the growth and killing of *Escherichia coli* and *Staphylococcus aureus*. The antibacterial capability of copper can be attributed to its inherent bactericidal effect on microorganisms by destroying bacterial cell

membranes and disrupting physiological processes inside bacteria. Copper-based additives have been proven to exert a dual-bactericidal effect due to the instantaneous Cu^{2+} ion release combined with a physical contact bactericide effect. As a result, the antibacterial activity remains substantial even against Gram-negative bacteria, which is associated with a compromised outer membrane that allows penetration of copper ions. Complete inhibition of bacterial growth in mortar specimens corroborates copper-based additives' broad-spectrum activity despite slow kinetics of Cu^{2+} ion release from the cementitious medium.

Zinc oxide (ZnO) showed a significantly greater inhibition zone against *S. aureus* than *E. coli*. This observation is attributed to the higher sensitivity of Gram-positive bacteria to zinc oxide-based antimicrobial agents due to simpler cell wall structures lacking an outer membrane. In the CFU mortar test, the difference in antibacterial efficacy against both strains was reduced because the slow kinetics of Zn^{2+} ions' release from the cementitious matrix lowered the ion concentration interacting directly with bacterial cells.

Eggshell powder (ESP) showed similar trends, with slightly larger inhibition zones of ESP against *E. coli* compared to *S. aureus*. In contrast to metal oxide materials, ESP and CaCO_3 -based additives exert antibacterial effects by altering the local environment's pH level and increasing the ionic activity of Ca^{2+} . While the outer membrane provides Gram-negative bacteria with protection against toxic metals and reactive oxygen species, it cannot inhibit the effect of the altered environmental pH level.

These results clearly demonstrate the effective implementation of waste-derived antibacterial materials in the development of functional cementitious additives with advanced antibacterial capabilities. It is evident that antibacterial functionality can be achieved using sustainable waste-derived antibacterial additives in cementitious construction applications, which highlights promising applications of antibacterial functionality in various construction sectors.

Table (16). Antibacterial Analysis Using (E. coli).

Antibacterial Analysis (E. coli)				
Sample	Type	Antibacterial Material	CFU (*10⁶ CFU/MI)	Observation
Control	Control Sample	No additive	56.8	High bacterial growth
B	Reference Mortar	No additive	56.6	Similar to control
A	Mortar & ZnO	ZnO	39.4	Moderate reduction
D	Mortar & Eggshell	ESP	0.2	Very strong reduction
C	Mortar & Copper Oxide	CuO	No growth	Complete inhibition

Table (17). Antibacterial Analysis Using (S. Aureus).

Antibacterial Analysis (S. aureus)				
Sample	Type	Antibacterial Material	CFU (10⁶CFU/MI)	Observation
Control	Control Sample	No additive	13.6	High bacterial growth
E	Reference Mortar	No additive	11.2	Slight reduction due to alkaline cement matrix
F	Mortar & ZnO	ZnO	4.8	Moderate reduction
H	Mortar & Eggshell	ESP	No growth	Complete inhibition
G	Mortar & Copper Oxide	CuO	No growth	Complete inhibition

Conclusions

The current research validates the potential of using sustainable waste-derived copper additives, ZnO, and eggshell powder as functional construction materials by investigating the antibacterial efficacy before and after integration within the epoxy matrix and cement-based materials. A comparative evaluation of conventional pure and waste-derived materials in powder form highlights the maintenance of sustained antibacterial functionality following incorporation in the epoxy system and cementitious composites. Waste-derived additives display similar or superior antibacterial capabilities compared to conventional pure counterparts. This study demonstrates that the selection of waste-derived antibacterial materials should consider bacterial strains and cell wall characteristics to achieve optimal antibacterial performance. The tests performed on the physical characteristics support the earlier conclusions related to the antibacterial activities of both systems. The epoxy coatings tested did not possess any porosity or absorption property thus confirming the assumption that they create a compact and impermeable medium minimizing the risk of moisture penetration and thus can be viewed as a feasible option concerning their antibacterial characteristics. The use of waste materials in cement-based compounds (especially CuO) allowed reducing both porosity and water absorption thanks to a filling effect preventing free spaces, which interrupts water-borne and air-borne bacteria infiltration. In general, the results confirm that the protective ability of both types of the systems depends not only on the ability of the additive to destroy bacteria but also on the efficiency of the structure obtained. . Among the tested waste-derived additives, copper additives present consistent and high-level antibacterial activities, resulting in full inhibition of *S. aureus* and *E. coli* bacterial growth within the cementitious system. Eggshell powders present a highly effective alternative to copper-based additives, presenting complete inhibition of *S. aureus* growth and effective inhibition of *E. coli*. On the other hand, ZnO presents considerable contributions to antibacterial performance in powder forms, moderate efficacy in epoxy, and limited efficacy within mortar, emphasizing its applicability in developing multifunctional construction materials. Therefore, this study suggests that the inclusion of biological waste such as eggs and industrial waste, including metallic compounds, can enable the creation of high-value multifunctional construction materials. Specifically, the development of multifunctional composites incorporating the investigated additives would lead to reduced environmental

impact, lower manufacturing cost, enhanced resistance to microbial corrosion, extended infrastructure lifespan, and increased safety. The produced materials could effectively be deployed in bio-aggressive conditions, e.g., hospitals, water treatment plants, sewers, and tunnels. Overall, this research lays the foundation for further exploration regarding the development of optimized mixtures and durability investigations in exposure conditions.

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