



## Enhanced Antibacterial Efficacy of Ag(I), Cu(II), and Zn(II) Modified Sodalite Zeolite Against *Escherichia coli* and *Staphylococcus aureus*

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<https://doi.org/10.14710/jksa.27.10.477-484>

### Article Info

#### Article history:

Received: 07<sup>th</sup> August 2024

Revised: 24<sup>th</sup> October 2024

Accepted: 28<sup>th</sup> October 2024

Online: 30<sup>th</sup> October 2024

#### Keywords:

sodalite; zeolite; antibacterial; disk diffusion; well diffusion

### Abstract

Sodalite zeolite modified with metal ions Ag<sup>+</sup>, Cu<sup>2+</sup>, and Zn<sup>2+</sup> was successfully synthesized and evaluated for antibacterial activity. The research aims to obtain silver, copper, and zinc metal-modified sodalite separately and determine their antibacterial activity on *Escherichia coli* and *Staphylococcus aureus* bacteria. Sodalite zeolite was synthesized using ludox and sodium aluminate through hydrothermal methods, ensuring uniform crystal growth and optimal crystallinity, as confirmed by X-ray diffraction (XRD) analysis. The average particle sizes of the modified zeolites were determined to be 54.9 nm for Ag-Zeolite, 37.2 nm for Cu-Zeolite, and 28.56 nm for Zn-Zeolite, with structural changes observed through alterations in peak intensity. Scanning Electron Microscopy - Energy Dispersive X-ray (SEM-EDX) analysis showed no significant change in the zeolite's morphology. In addition, the EDX results showed the presence of Ag (3.15%), Cu (3%), and Zn (2.41%) metals indicating successful ion exchange. Antibacterial assays revealed that Cu-Zeolite demonstrated superior efficacy inhibition zones against *Escherichia coli* (14.04±1.26) and *Staphylococcus aureus* (20.74±0.48), highlighting its potential as an antimicrobial agent. The mechanism of action involved the controlled release of metal ions, disrupting bacterial cell membranes and metabolic processes. Notably, Cu<sup>2+</sup> ions exhibited the strongest antibacterial properties due to their smaller ionic radius and higher electronegativity than Ag<sup>+</sup> and Zn<sup>2+</sup>. This research underscores the promising applications of metal-ion-modified sodalite zeolite in medical and environmental contexts.

### 1. Introduction

Developing materials capable of inhibiting microbial growth is very interesting in the scientific field. This is because of its various applications, such as water filter systems, textiles, and medicines. Recently, much attention has been paid to a reliable and more affordable type of antibacterial, namely modified zeolite-based components [1]. Zeolite is a microporous aluminosilicate mineral generally used as an adsorbent, commercial catalyst, and cation exchanger. One of the factors that influences the type of zeolite is the ratio between silica

and alumina, where this ratio will affect the physical properties of the resulting zeolite [2].

Sodalite is a synthesized zeolite with a low Si/Al ratio. Sodalite is known as a host molecule material in other synthesized zeolite framework structures, such as type A zeolite and faujasite [3]. The hydrothermal method is one method that is often used to synthesize zeolites [4]. The hydrothermal method is a method that does not require high costs and has easy stages in making zeolites and nanomaterials. Another advantage is that it can produce nanoparticle crystals at low temperatures and make the nanoparticles evenly dispersed [5].

Zeolite itself lacks antibacterial properties. However, it gains antibacterial capabilities when modified with specific metal ions, making zeolites a highly effective material for antibacterial applications. Zeolite modification can be carried out through the process of exchanging  $\text{Na}^+$  ions in the zeolite with other metals as antibacterial agents. Antimicrobial metals include silver, copper, zinc, mercury, lead, nickel, and cadmium [6]. Silver has a broad spectrum as an antimicrobial agent with low toxicity to humans [7]. High copper levels in cells can cause damage to membrane integrity [8]. Zinc is also widely used as an antimicrobial agent and has been widely used as an inhibitor of cariogenic bacteria in teeth [9].

Several studies on using zeolite as an antibacterial material have been carried out previously. Yao *et al.* [10] carried out an antibacterial test of zeolite Li *et al.* [11] conducted an antibacterial test on zeolite modified with  $\text{Ag}^+$  ions and obtained a minimum inhibitory concentration against *Escherichia coli* bacteria of 9.17 mg/L, while for *Staphylococcus aureus* bacteria, it was 12.50 mg/L. Milenkovic *et al.* [12] conducted an antibacterial test of zeolite A modified with  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Ag}^+$  ions against *Escherichia coli* bacteria and found that Ag-zeolite had the best inhibitory ability.

Based on this explanation, this research aims to obtain synthesized zeolite sodalite nanoparticle type with a low Si/Al ratio modified with metal ions ( $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ag}^+$ ) and determine the antibacterial ability of synthesized zeolite modified by  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ag}^+$  ions, using the disc diffusion and well diffusion methods against *Escherichia coli* and *Staphylococcus aureus* bacteria. This research introduces the novel use of zeolite loaded with various metals, including Ag, Cu, and Zn, for antibacterial applications. The combination of these metals on a zeolite framework represents an innovative approach, offering enhanced antibacterial properties that distinguish this study from existing research in the field.

## 2. Experimental

### 2.1. Materials and Tools

LUDOX HS-40 ( $\text{SiO}_2 \cdot 5\text{H}_2\text{O}$ ) (Sigma-Aldrich), sodium aluminate ( $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3$ ) (Sigma-Aldrich), sodium hydroxide (NaOH) (Merck), distilled water, silver nitrate ( $\text{AgNO}_3$ ) (Merck), copper (II) nitrate trihydrate ( $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ ) (Merck), zinc nitrate hexahydrate ( $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ) (Sigma-Aldrich), *Escherichia coli* (*E. coli*) bacteria, *Staphylococcus aureus* (*S. aureus*) bacteria, ethanol 70% (Merck), nutrient agar (Merck), nutrient broth (Merck), chloramphenicol (CHL, Kimia Farma).

Glassware (Pyrex), magnetic stirrer (Cimarec), oven (Binder ed-53), analytical balance (Ohaus), inoculation needle, cork borer, spirit burner, petri dish (Anumbra), spreader, micropipette (Thermo Finn timer), incubator, laminar air flow (LAF) (E-Scientific), orbital shaker (Thermo MaxQ-2000), UV-Vis spectrophotometer (Shimadzu UV-1201), X-ray diffraction (XRD) (Shimadzu XRD-7000), and scanning electron microscope – energy dispersive X-ray (SEM-EDX) (Jeol JSM-6510LA).

### 2.2. Synthesis of Zeolite

The synthesis of zeolite in this research was based on previous studies by Demirci *et al.* [13] and Osonio and Vasquez [14]. Zeolite was synthesized hydrothermally with a Si/Al ratio of 1. To prepare a 1.69 M NaOH solution, 17.45 g of NaOH pellets were dissolved in 249 mL of distilled water. Afterward, 16.4 g of  $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3$  was added to the NaOH solution and stirred until fully dissolved, resulting in a 0.75 M  $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3$  solution. Then, 11.6 mL of Ludox was added to this solution and stirred until homogeneous. The hydrothermal process was conducted in an oven at 150°C for 24 hours, followed by an aging process at room temperature for 24 hours. The mixture was then filtered, washed until neutral, and dried. The synthesized zeolite solids were characterized using SEM-EDX and XRD.

### 2.3. Modification of Synthesized Zeolite

The enrichment of silver, copper, and zinc metals into zeolite was based on the methods of Cui *et al.* [15] and Septommy *et al.* [16]. A 2 g sample of synthesized zeolite was combined with 20 mL of 0.05 M  $\text{AgNO}_3$  solution. After 5 hours, the mixture was filtered, and the solid was washed with distilled water and dried at 80°C for 3 hours, yielding  $\text{Ag}^+$ -Zeolite.

The same procedure was followed to produce  $\text{Cu}^{2+}$ -Zeolite and  $\text{Zn}^{2+}$ -Zeolite by adding 0.05 M  $\text{Cu}(\text{NO}_3)_2$  and 0.05 M  $\text{Zn}(\text{NO}_3)_2$  solutions, respectively. The modified zeolite samples were then characterized using SEM-EDX and XRD.

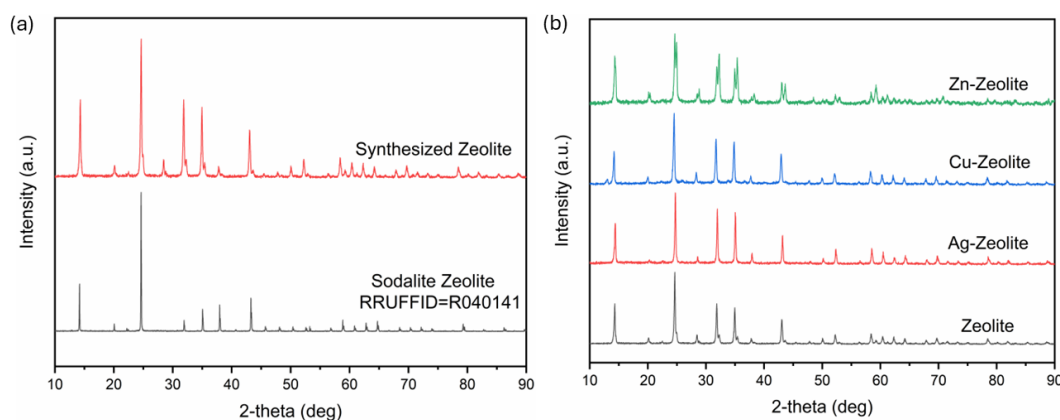
### 2.4. Antibacterial Activity Test of Modified Zeolite

The initial step involved preparing the MacFarland Standard Solution, nutrient agar, and nutrient broth media. *E. coli* and *S. aureus* bacteria were rejuvenated, and bacterial suspensions were prepared. The antibacterial test was conducted using the disc diffusion method, with Whatman filter paper No. 42 as the disc material. Paper discs dipped in the sample solution were placed on agar media inoculated with 0.1 mL of either *S. aureus* or *E. coli* bacterial suspension. Incubation was carried out at 37°C for 24 hours. Following incubation, the diameter of the inhibition zone was measured with a caliper to obtain the average clear zone diameter. CHL was used as a positive control, while distilled water was a negative control. Control antibacterial tests were also performed on 0.5 M  $\text{AgNO}_3$ , 0.5 M  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ , and  $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  solutions.

## 3. Results and Discussion

### 3.1. Synthesis and Modification of Zeolite

Synthesized zeolite can be prepared from materials that serve as sources of silica and alumina, such as ludox ( $\text{SiO}_2 \cdot 5\text{H}_2\text{O}$ ) and sodium aluminate ( $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3$ ). Ludox, a colloidal form of silica, is chosen as the silica source due to its high concentration of silanol functional groups and large surface area. These properties make ludox an effective binder for metals, oxides, and other silica-based materials, while its reactive functional groups enhance its performance in the synthesis process [17].



**Figure 1.** Diffractogram of (a) the standard and synthesized zeolite before modification and (b) zeolite after modification

The synthesis of zeolite in this context is carried out using the hydrothermal method, which involves combining the precursors in a molar ratio of  $\text{Na}_2\text{O}:\text{Al}_2\text{O}_3:\text{SiO}_2:150\text{ H}_2\text{O}$ . This method promotes the formation of zeolite crystals under controlled conditions. The hydrothermal process is essential for ensuring uniform and complete crystal growth, while the aging process enhances the crystallinity of the zeolite, optimizing crystal formation.

Zeolite formation occurs in alkaline conditions, as pH is crucial in determining the species of silicates and aluminates present in the solution. Specifically, the  $\text{Al}(\text{OH})_4^-$  anion is the primary zeolite-forming aluminate species, with its concentration reaching optimal levels at  $\text{pH} \geq 9$ . Similarly, silicate species, such as  $\text{Si}(\text{OH})_4$  and the  $\text{Si}^{4+}$  cation, exhibit their highest concentrations at  $\text{pH} > 12$  [18]. Additionally, sodium ions ( $\text{Na}^+$ ) act as balancing cations within the zeolite structure. Since the  $\text{Al}^{3+}$  ions in the zeolite framework generate excess negative charges,  $\text{Na}^+$  ions are necessary to neutralize these charges, thereby stabilizing the overall structure [19]. The interaction between sodium aluminate and ludox leads to the polymerization of aluminate and silicate species, resulting in the formation of a white aluminosilicate gel. This gel indicates a strong interaction between the aluminate and silicate components, which is critical for the successful synthesis of zeolite [20].

The hydrothermal process aims to achieve uniform and optimal crystal growth. The aging process enhances the zeolite's crystallinity, maximizing crystal formation. During the hydrothermal method, the alumina silicate mixture undergoes rearrangement, leading to a more regular structure and crystal formation [21]. The formed crystals consist of bonds between Si-O-Al atoms, with oxygen atoms as links [22]. Different metal cations can be introduced to create functionalized zeolite materials. For example, Ag<sup>+</sup>-Zeolite (Ag-Zeolite) is synthesized by adding a silver nitrate ( $\text{AgNO}_3$ ) solution to synthesized zeolite. Similarly, Zn<sup>2+</sup>-Zeolite (Zn-Zeolite) is produced by incorporating zinc nitrate ( $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ), and Cu<sup>2+</sup>-Zeolite (Cu-Zeolite) is obtained by adding copper nitrate ( $\text{Cu}(\text{NO}_3)_2$ ). These metal precursors donate Ag<sup>+</sup>, Zn<sup>2+</sup>, and Cu<sup>2+</sup> cations, which replace Na<sup>+</sup> ions in the zeolite framework.

**Table 1.** The 2θ values of synthesized zeolite and standard zeolite sodalite

2θ value of sodalite zeolite standard (°)	Intensity	2θ synthesized zeolite (°)	Intensity of synthesized zeolite
14.14	53.18	14.34	53
20.05	15.24	20.16	8
24.62	100	24.65	100
28.51	19.8	28.47	12
31.96	98.07	31.86	58
35.10	77.6	34.95	53
38.02	13.65	37.81	7
43.35	19.89	43.05	37

### 3.2. Characteristics of Zeolite and Modified Zeolite

#### 3.2.1. Crystal Structures of Zeolite and Modified Zeolite

XRD analysis is used to determine the crystal phase or type of the zeolite and its degree of crystallinity. The XRD diffractograms of the synthesized zeolite, both before and after modification with Ag(I), Cu(II), and Zn(II) metal ions, are presented in Figure 1. As shown in Figure 1a, the synthesized zeolite exhibits a crystalline phase characteristic of the sodalite type. The average crystal size of the unmodified zeolite is approximately 47.6 nm, classifying it as sodalite nanoparticles (NPs) due to its nanoscale dimensions and structural properties. The mean crystal size was calculated using the Debye-Scherrer equation.

As depicted in Figure 1b and summarized in Table 1, the diffractogram of the unmodified synthesized zeolite shows distinct peaks associated with sodalite zeolite at 2θ values of 14.34°, 20.16°, 24.65°, 28.47°, 31.86°, 34.95°, 37.81°, and 43.05°. These peaks confirm the crystalline structure of the sodalite phase (RRUFFID=R040141). After modification with metal ions, the average particle sizes of the resulting zeolite composites were found to be 54.9 nm for Ag-Zeolite, 37.2 nm for Cu-Zeolite, and 28.56 nm for Zn-Zeolite.

**Table 2.** Results of EDX analysis of synthesized sodalite zeolite before and after modification

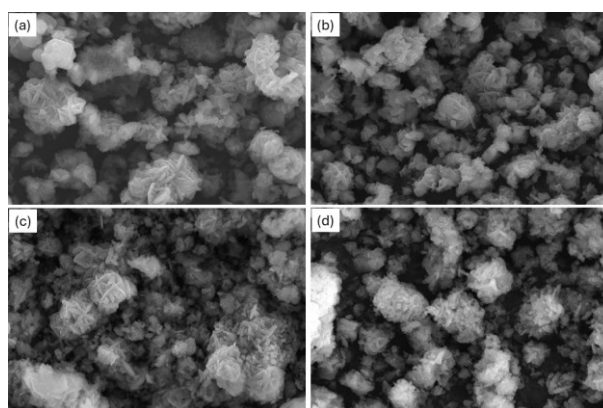
Elemental	Massa (%)			
	Zeolite Sodalite	Ag-Zeolite	Cu-Zeolite	Zn-Zeolite
Si	10.59	8.24	11.48	8.57
Al	11.61	9.54	12.49	9.55
Na	11.67	9.07	10.29	8.76
Ag	-	3.15	-	-
Cu	-	-	3.00	-
Zn	-	-	-	2.41
O	66.11	70.01	62.74	70.72
Ratio Si/Al	1.1	1.16	1.09	1.11

The incorporation of  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$  ions results in a noticeable reduction in the intensity of several 2 $\theta$  peaks in the diffractogram. This decrease in peak intensity is likely due to interference from these metal ions, as the reflected X-rays from the crystal planes are partially blocked by the  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$  ions attached to the zeolite structure. Additionally, modification with  $\text{Cu}^{2+}$  ions introduces a new peak at  $2\theta = 12.93^\circ$ , which was not present in the unmodified zeolite. Similarly, modification with  $\text{Zn}^{2+}$  ions leads to the appearance of a new peak at  $2\theta = 53.02^\circ$ , further confirming the structural changes induced by these metal ions.

### 3.2.2. Surface Morphology of Zeolite and Modified Zeolite

SEM-EDX analysis was performed to examine the surface morphology and elemental composition of the synthesized zeolite. The results, presented in Figure 2, show that the synthesized zeolite exhibits a flat, clustered, spherical crystal structure characteristic of sodalite. After modification with metal ions such as  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$ , the morphology of the sodalite remains largely unchanged, suggesting that introducing these metal ions does not significantly alter the crystal structure. SEM-EDX analysis further confirms that the modification process does not substantially affect the overall morphology, indicating the stability of the sodalite crystal structure. Elemental composition analysis via EDX reveals the primary constituents of both the unmodified and modified sodalite zeolite, as summarized in Table 2, showing the presence of elements from both the sodalite framework and the introduced metal ions. These findings confirm successful modification without structural disruption.

As shown in Table 2, the synthesized sodalite zeolite exhibits a Si/Al ratio ranging from 1.09 to 1.16, indicating a balanced structural composition typical of sodalite-type zeolites. The presence of Ag, Cu, and Zn elements in the modified samples confirms the successful incorporation of  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$  ions into the sodalite framework. A notable decrease in the sodium (Na) mass percentage after modification suggests that  $\text{Na}^+$  ions were exchanged with the introduced  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$  ions during the ion exchange process. Specifically, Ag-Zeolite contains 3.15% Ag by mass, Cu-Zeolite contains 3% Cu, and Zn-Zeolite contains 2.41% Zn.

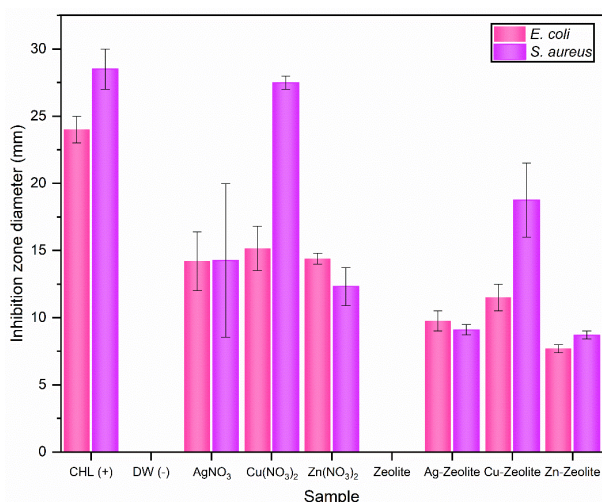


**Figure 2.** Morphology images of (a) sodalite zeolite before modification, (b) Ag-Zeolite, (c) Cu-Zeolite, (d) Zn-Zeolite at 3,000 $\times$  magnification

The higher concentration of  $\text{Ag}^+$  ions in the sodalite structure, compared to  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  ions, can be attributed to the ease with which  $\text{Ag}^+$  is exchanged for  $\text{Na}^+$  ions. Despite  $\text{Ag}^+$  having a relatively larger ionic radius (127 pm) than  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ , its size is closer to that of  $\text{Na}^+$  ions, which have an ionic radius of 116 pm. This size similarity facilitates a smoother ion-exchange process [23]. Additionally, the identical +1 charge of  $\text{Ag}^+$  and  $\text{Na}^+$  (or other  $\text{M}^+$  ions) enhances the exchange, minimizing the energetic barriers typically associated with charge imbalance during ion substitution [24]. These factors—the similarity in ionic size and identical charge—allow  $\text{Ag}^+$  ions to replace  $\text{Na}^+$  ions more efficiently in the sodalite framework than  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ , whose differing charges and ionic radii make the exchange process less favorable.

### 3.3. Antibacterial Activity of Zeolite and Modified Zeolites

The antibacterial activity of sodalite zeolite modified with Ag(I), Cu(II), and Zn(II) metal ions was evaluated against *E. coli* (a gram-negative bacterium) and *S. aureus* (a gram-positive bacterium). The bacterial cultures were standardized to the McFarland 0.5 standard, corresponding to approximately  $1.5 \times 10^8$  CFU/mL, ensuring consistent bacterial colony numbers across all tests. CHL, a well-known antibiotic, was used as the positive control, while distilled water was the negative control. Additionally, 0.05 M solutions of  $\text{AgNO}_3$ ,  $\text{Cu}(\text{NO}_3)_2$ , and  $\text{Zn}(\text{NO}_3)_2$  were used as control samples for their respective metal ions.

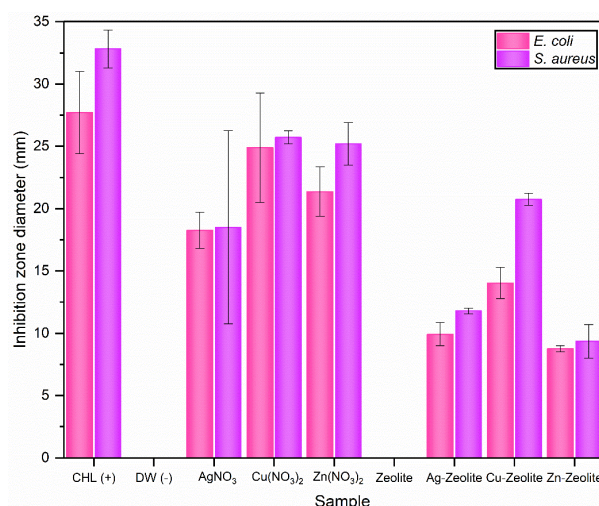


**Figure 3.** Antibacterial activity using the disc diffusion method against *E. coli* and *S. aureus* bacteria

The disk diffusion method was employed to assess antibacterial activity based on the principle that a disk saturated with an antibacterial agent diffuses radially through the agar medium, creating a clear zone of bacterial inhibition around the disk [25]. After incubation, a clear zone forms around the disk if the compound effectively inhibits bacterial growth. Figure 3 indicates that CHL, as the positive control, exhibited the largest clear zone diameter, demonstrating its potent antibacterial effect on both *E. coli* and *S. aureus*.

Distilled water, as the negative control, and the unmodified zeolite did not produce any clear zones, confirming their lack of antibacterial properties. Among the modified zeolites, Cu-Zeolite exhibited the largest average clear zone diameter for *E. coli* and *S. aureus*, outperforming Ag-Zeolite and Zn-Zeolite. Similarly, in the control samples, the Cu(NO<sub>3</sub>)<sub>2</sub> solution produced the largest clear zone diameter compared to AgNO<sub>3</sub> and Zn(NO<sub>3</sub>)<sub>2</sub> solutions, indicating that copper ions exert the strongest antibacterial effect. The well-diffusion method operates similarly to the disk diffusion method. In this approach, the antimicrobial agent is introduced into wells created on an agar medium inoculated with the test microorganism. As the antimicrobial agent diffuses through the agar, it inhibits the growth of the microbes in the surrounding area. The size of the clear zone around the well indicates the efficacy of the antimicrobial agent in inhibiting microbial growth [26].

Figure 4 shows that CHL, the positive control, had the largest clear zone, confirming its strong antibacterial effect against *E. coli* and *S. aureus*. The results from the well-diffusion method also reveal that neither distilled water nor unmodified zeolite generated a clear zone, indicating that they lack antibacterial properties and are ineffective in inhibiting the growth of *E. coli* and *S. aureus*. Among the modified zeolites, Cu-Zeolite exhibited the strongest antibacterial activity, producing the largest clear zone in both bacteria, surpassing the effects of Ag-Zeolite and Zn-Zeolite. Similarly, the Cu(NO<sub>3</sub>)<sub>2</sub> solution also produced the largest clear zone than AgNO<sub>3</sub> and Zn(NO<sub>3</sub>)<sub>2</sub>, further confirming the superior antibacterial effectiveness of copper ions in this context.



**Figure 4.** Antibacterial activity using the well-diffusion method against *E. coli* and *S. aureus* bacteria

Ag-Zeolite, Cu-Zeolite, and Zn-Zeolite exhibit lower antibacterial activity compared to their respective control solutions (AgNO<sub>3</sub>, Cu(NO<sub>3</sub>)<sub>2</sub>, and Zn(NO<sub>3</sub>)<sub>2</sub>). This disparity arises because the control solutions can directly interact with the bacterial cells, resulting in larger clear zones. In contrast, when metal ions are loaded into zeolite, they are released more gradually, leading to slower interaction with the bacteria. Zeolites modified with antibacterial metal ions, such as Ag<sup>+</sup>, Cu<sup>2+</sup>, or Zn<sup>2+</sup>, can still inhibit bacterial growth, but through a different mechanism. This mechanism involves the controlled release of the metal ions from the zeolite framework, contributing to the antibacterial action.

The slow release of Ag<sup>+</sup>, Cu<sup>2+</sup>, and Zn<sup>2+</sup> ions from the zeolite allows these ions to interact with the negatively charged bacterial cell membranes. This interaction disrupts the integrity of the bacterial cell walls in both *E. coli* (gram-negative) and *S. aureus* (gram-positive), causing cell wall damage [27]. Additionally, Cu<sup>2+</sup> ions can penetrate the bacterial cell protoplasm and form Cu-protein compounds, which interfere with the cell's metabolic processes, ultimately inhibiting its ability to function and reproduce [28].

In this study, the antibacterial mechanism appears to be primarily attributed to the release of Ag<sup>+</sup>, Cu<sup>2+</sup>, and Zn<sup>2+</sup> ions from the zeolite surface, as the bacterial cells are too large to enter the pores of the zeolite. Both *E. coli* and *S. aureus* bacteria adhere to the surface of the zeolite, where they interact with the released metal ions. This interaction is crucial to the observed antibacterial activity, as the ions bind to the bacteria's cell membranes, causing damage through the oligodynamic effect [29].

The release of Ag<sup>+</sup>, Cu<sup>2+</sup>, and Zn<sup>2+</sup> ions from the zeolite is facilitated by their interactions with the sulfhydryl (R-SH) groups on the bacterial cell membranes. The oligodynamic effect, which refers to the ability of small amounts of metal ions to exert a potent antimicrobial effect, is activated during this process. Metal ions, such as Ag<sup>+</sup>, Cu<sup>2+</sup>, and Zn<sup>2+</sup>, exhibit antibacterial properties by coagulating proteins and reacting with sulfhydryl groups, rendering these groups inactive [30].

According to the Hard and Soft Acid Base (HSAB) theory, the sulfhydryl group (R-SH) is classified as a soft base, while  $\text{Ag}^+$  is categorized as a soft acid [31]. Soft acids, such as  $\text{Ag}^+$ , have a small ionic radius, low oxidation state, and strong polarization, allowing them to bind effectively with soft bases like sulfhydryl groups. In contrast,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  ions are considered borderline acids but still exhibit significant antibacterial properties through similar interactions [32].

The number of metal ions embedded in zeolite significantly influences its antibacterial effectiveness in inhibiting the growth of *E. coli* and *S. aureus* bacteria. Higher concentrations of  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$  ions enhance the antibacterial activity. This occurs due to the electrostatic interaction between the positively charged metal ions and the negatively charged bacterial cell membrane, which creates a strong attraction between them [33]. As more  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$  ions are released from the zeolite, more bacterial cells are targeted and killed, forming larger clear zones during antibacterial tests. The antibacterial mechanism of metal ions involves their interaction with critical bacterial components such as the peptidoglycan cell wall, bacterial DNA, and bacterial proteins.

The interaction between metal ions and the peptidoglycan cell wall and plasma membrane results in the disruption of these structures, ultimately leading to cell lysis [34]. Metal ions can also bind to bacterial DNA, interfering with its ability to replicate. By binding to DNA bases, the metal ions inhibit bacterial reproduction through the process of binary fission [35]. Additionally, metal ions can interfere with protein synthesis by interacting with bacterial ribosomes (specifically the 70S ribosomes), thereby disrupting protein production and compromising the integrity of the plasma membrane [36].

Among the metal-ion-modified zeolites, sodalite zeolite modified with  $\text{Cu}^{2+}$  ions exhibited the most potent antibacterial activity, producing the largest clear zones. This can be explained by the size and electronegativity of the  $\text{Cu}^{2+}$  ions compared to  $\text{Ag}^+$  and  $\text{Zn}^{2+}$  ions. The smaller ionic radius of  $\text{Cu}^{2+}$  (72 pm) allows for stronger interactions with bacterial cell components compared to the larger radii of  $\text{Ag}^+$  (113 pm) and  $\text{Zn}^{2+}$  (83 pm) ions [37].

This smaller size enhances the attraction between  $\text{Cu}^{2+}$  ions and the negatively charged bacterial cell membrane. Additionally,  $\text{Cu}^{2+}$  has a relatively high electronegativity of 1.90, contributing to its strong binding with the bacterial membrane. In contrast,  $\text{Ag}^+$  ions, despite having a similar electronegativity (1.93), are more prone to reduction [38]. The  $\text{Ag}^+$  ion easily loses its antimicrobial activity due to its tendency to undergo reduction from  $\text{Ag}^+$  to  $\text{Ag}^0$ , which diminishes its antibacterial properties.  $\text{Zn}^{2+}$  ions, with a lower electronegativity of 1.65, exhibit weaker antibacterial activity compared to  $\text{Cu}^{2+}$  ions [39, 40].

The experimental results demonstrate that sodalite zeolite loaded with metal ions, particularly  $\text{Cu}^{2+}$  exhibits strong antibacterial properties. Notably, these metal-ion-loaded zeolites show greater efficacy against

*S. aureus* (Gram-positive bacteria) than *E. coli* (Gram-negative bacteria). The difference in susceptibility can be attributed to the structural complexity of the bacterial cell walls. Gram-positive bacteria, like *S. aureus*, have a simpler cell wall structure, making them more vulnerable to metal ion interactions. In contrast, Gram-negative bacteria, such as *E. coli*, possess an additional outer membrane composed of lipopolysaccharides, which act as a barrier to prevent the penetration of metal ions. This protective layer contributes to the reduced sensitivity of Gram-negative bacteria to metal-ion-based antibacterial agents [41, 42]. Thus, the combination of smaller ionic radius, higher electronegativity, and efficient interaction with bacterial cells makes Cu-modified zeolite a particularly effective antimicrobial agent, outperforming its Ag and  $\text{Zn}^{2+}$  counterparts.

#### 4. Conclusion

This study investigated the synthesis, modification, and antibacterial activity of sodalite zeolite loaded with metal ions— $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$ . The zeolite was synthesized using Ludox and sodium aluminate via hydrothermal methods, achieving uniform crystal growth and optimal crystallinity, confirmed by XRD analysis. Post-modification, Ag-Zeolite, Cu-Zeolite, and Zn-Zeolite exhibited average particle sizes of 54.9 nm, 37.2 nm, and 28.56 nm, respectively, with changes in peak intensity indicating structural alterations. SEM-EDX analysis showed no significant change in the zeolite's morphology, confirming successful ion exchange with elemental analysis. Antibacterial efficacy was evaluated against *E. coli* and *S. aureus* using the disk diffusion method, revealing that Cu-Zeolite exhibited the greatest antibacterial activity. The mechanism primarily involved gradually releasing metal ions, disrupting bacterial cell membranes and metabolic processes.  $\text{Cu}^{2+}$  ions, with their smaller ionic radius and higher electronegativity, were the most effective against both bacterial strains, outperforming  $\text{Ag}^+$  and  $\text{Zn}^{2+}$ . This research highlights the potential of metal-ion-modified sodalite zeolite as an effective antimicrobial agent, suggesting avenues for further applications in medical and environmental fields.

#### Acknowledgment

We sincerely thank the Dean of the Faculty of Science and Mathematics at UNDIP for supporting this research through funding for the 2023 fiscal year, as outlined in contract No. 21/UN7.F8/PP/II/2023.

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