

Synthesis, Characterization, and Biological Activity of sulfamethoxazole-Based Metal Complexes: Antibacterial and Antifungal Evaluation

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ABSTRACT

This study investigates the synthesis, characterization, and biological activity of sulfamethoxazole (SMX)-based metal complexes (SMX_Cu, SMX_Co, SMX_Cd, and SMX_Mn) against various bacterial and fungal pathogens. The complexes were synthesized and characterized using FTIR spectroscopy, which confirmed metal-ligand coordination. The antimicrobial activity of the complexes was evaluated through zone of inhibition (ZOI) assays, minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), and antibiofilm activity tests. The results showed that SMX_Mn and SMX_Cd complexes exhibited the strongest antibacterial and antifungal activities, particularly against *Aspergillus niger*, *Aspergillus oryzae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus licheniformis*. These complexes also demonstrated significant antibiofilm activity, especially at higher concentrations. The FTIR analysis confirmed the successful coordination of metal ions, which likely enhanced the antimicrobial properties of SMX. The findings suggest that SMX-metal complexes, particularly SMX_Mn and SMX_Cd, have potential as effective antimicrobial and antibiofilm agents and may serve as valuable candidates for the treatment of resistant infections.

Keywords: Sulfamethoxazole, Antifungal, Antibacterial, Antibiofilm, Biofilm Inhibition

Introduction

Sulfamethoxazole (SMX) is a widely used sulfonamide antibiotic. It acts by inhibiting bacterial folic acid synthesis, which is essential for bacterial growth. SMX is frequently used in combination with trimethoprim to treat a variety of infections caused by both Gram-positive and Gram-negative bacteria. However, the widespread use of SMX has led to the development of resistance in several bacterial species, reducing its effectiveness. Antimicrobial resistance (AMR) is one of the greatest global health threats of the 21st century. The development of resistant strains has made it increasingly difficult to treat common infections, contributing to higher healthcare costs and greater morbidity and mortality rates (Gal et al., 2015).

Given the rise of resistant pathogens, there is a growing need for novel antimicrobial agents. One promising approach involves the use of metal-based complexes in conjunction with antibiotics. Metal ions, such as silver (Ag), copper (Cu), zinc (Zn), and nickel (Ni), have inherent antimicrobial properties and can enhance the activity of antibiotics by altering their mechanisms of action. The combination of antibiotics like SMX with metal ions results in metal-drug complexes that exhibit enhanced stability, solubility, and antimicrobial activity. These complexes can interact with bacterial membranes, disrupt cellular processes, and generate reactive oxygen species (ROS), all of which contribute to their increased antimicrobial effectiveness (Yang et al., 2019).

The interaction between metal ions and sulfonamides like SMX can enhance the drug's ability to target microorganisms. When SMX binds to metal ions, it forms stable complexes that can exhibit stronger antibacterial and antifungal activity than the free drug. For example, SMX complexes with silver or copper ions have been shown to possess increased antibacterial activity against resistant strains of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. These complexes also demonstrate antifungal activity against fungi like *Candida albicans* and *Aspergillus niger*, indicating their potential use in treating a broader range of infections (Jousset et al., 2018).

The antimicrobial properties of metal-based complexes are believed to arise from the combined effects of the metal and the sulfonamide. Metals like copper and silver possess intrinsic antimicrobial effects, which can synergize with the antibiotic action of SMX (Sharma et al., 2022). For instance, silver ions have long been known for their ability to disrupt microbial cell membranes, interfere with microbial DNA replication, and generate ROS, all of which contribute to the killing of bacterial and fungal cells. Copper ions, similarly, can interfere with cellular respiration and protein synthesis, further enhancing the antimicrobial activity of the complex. When combined with SMX, these metal ions can offer a multi-targeted approach that may be more effective than traditional single-target antibiotics (Salah et al., 2021).

The synthesis of SMX-based metal complexes requires careful selection of metal ions and appropriate reaction conditions to ensure the formation of stable, bioactive complexes. Techniques such as sol-gel, hydrothermal synthesis, and solvent evaporation are commonly used to prepare these complexes. Once synthesized, the complexes must be characterized using various spectroscopic and analytical techniques, such as UV-Vis spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR), and X-ray diffraction (XRD).

These methods help determine the coordination environment of the metal ion and the interactions between SMX and the metal, providing insight into the structure-activity relationship of the complexes. Characterization is critical in ensuring the stability and bioavailability of the metal-drug complexes, as well as their potential therapeutic value (Yang et al., 2019).

After synthesis and characterization, the antimicrobial activity of SMX-based metal complexes is typically evaluated using standard microbiological methods. The minimum inhibitory concentration (MIC) is commonly used to determine the efficacy of these complexes against bacterial and fungal pathogens. In addition, disk diffusion assays and broth dilution methods can be employed to assess the spectrum of activity and the potential for synergy between SMX and the metal ion. Studies have shown that SMX-metal complexes exhibit improved antimicrobial properties compared to free SMX, suggesting that metal coordination enhances the overall effectiveness of the drug (Liao et al., 2020).

This manuscript aims to investigate the synthesis, characterization, and antimicrobial activity of SMX-based metal complexes. Specifically, we focus on the preparation of SMX complexes with copper, silver, and nickel ions. We evaluate the antibacterial activity of these complexes against resistant and non-resistant bacterial strains and assess their antifungal activity. We also compare the antimicrobial efficacy of the metal complexes to that of free SMX to determine whether metal coordination enhances its activity. By exploring the potential of SMX-metal complexes, we hope to contribute to the development of new, more effective antimicrobial therapies that can combat the growing problem of antibiotic resistance.

Materials and Methods

Ligand Preparation

The SMX-metal complexes were synthesized by reacting SMX with metal salts, including copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), cobalt chloride (CoCl_2), cadmium chloride (CdCl_2), and manganese chloride (MnCl_2). To prepare each complex, 0.5 mmol of SMX was dissolved in 10 mL of ethanol, and a corresponding metal salt solution (0.5 mmol) in 10 mL of distilled water was added dropwise under constant stirring following Qamar et al. (2024). The pH was adjusted to 7.0 using dilute sodium hydroxide, and the mixture was stirred for 4-6 hours at room temperature to allow for complexation. After the reaction, the solution was filtered to remove any unreacted materials, and the solvent was evaporated under reduced pressure. The resulting solid complexes were washed with cold ethanol, filtered, and dried under vacuum. Each complex was then purified by recrystallization from a mixture of ethanol and acetone. The complexes were characterized using UV-Vis spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy to confirm the metal coordination and structure.

FTIR Spectroscopy

FTIR spectroscopy was used to identify the functional groups involved in the coordination between SMX and the metal ions in the complexes. The synthesized SMX-metal complexes were finely ground with potassium bromide (KBr) and pressed into pellets. FTIR spectra were recorded in the range of 4000 to 400 cm^{-1} using an

FTIR spectrometer (Iram Liaqat et al., 2025). The characteristic absorption bands corresponding to the functional groups of SMX (such as the sulfonamide group) and the metal-ligand coordination were analyzed to confirm the binding of the metal ions to the SMX molecule. Shifts in the functional group peaks compared to the free SMX were noted to assess the changes in the electronic environment due to metal coordination.

Antifungal Activity

The antifungal activity of the synthesized SMX-metal complexes was evaluated using the agar well diffusion method against *A. oryzae* and *A. niger* following Muhammad et al. (2025). Agar plates were prepared by spreading a uniform suspension of the fungal culture on the surface of the agar medium. Wells of 6 mm diameter were punched into the agar, and 100 μ L of each complex solution at concentrations of 50 μ g/mL, 25 μ g/mL, and 12.5 μ g/mL were added to the wells. A control well was also prepared using voriconazole at a concentration of 50 μ g/mL as the positive control. The plates were incubated at 28°C for 48-72 hours. The antifungal activity was assessed by measuring the zone of inhibition (ZOI) around the wells in millimeters (mm). The ZOI was recorded to evaluate the effectiveness of each complex at different concentrations, and the results were compared to the control.

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the synthesized SMX-metal complexes against *Aspergillus oryzae* and *Aspergillus niger* were determined using the tube dilution method (Muhammad et al., 2025). A series of twofold dilutions of each complex were prepared in sterile broth to achieve final concentrations of 60 μ g/mL - 2 μ g/mL, and lower concentrations. Each test tube was inoculated with a fungal suspension to achieve a final concentration of approximately 1×10^6 spores/mL. The tubes were incubated at 28°C for 48 hours. The MIC was determined as the lowest concentration of the complex that completely inhibited visible fungal growth, as assessed by turbidity. To determine the MFC, 100 μ L from each tube showing no visible growth was subcultured onto fresh agar plates and incubated for 48 hours. The MFC was recorded as the lowest concentration where no fungal growth was observed on the agar plate. Both the MIC and MFC were compared to the control, which contained the same fungal inoculum but without the complex.

Antibacterial Activity

The antibacterial activity of the synthesized SMX-metal complexes was assessed using the agar well diffusion method against *E. coli*, *P. aeruginosa*, and *B. licheniformis* following (Iram Liaqat et al., 2023). Agar plates were inoculated with 100 μ L of bacterial suspension (approximately 10^6 CFU/mL) spread uniformly on the surface. Wells of 6 mm diameter were created in the agar, and 100 μ L of each complex solution at concentrations of 50 μ g/mL, 25 μ g/mL, and 12.5 μ g/mL were added to the wells. Rifampicin (50 μ g/mL) was used as a positive control, and a well with no antimicrobial agent served as the negative control. The plates were incubated

at 37°C for 24 hours. The antibacterial activity was evaluated by measuring the zone of inhibition (ZOI) in millimeters (mm) around each well. The ZOI was recorded to assess the effectiveness of each complex at the different concentrations, and the results were compared to the control.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the synthesized SMX-metal complexes against *E. coli*, *P. aeruginosa*, and *B. licheniformis* were determined using the tube dilution method (I. Liaqat et al., 2025). A series of twofold dilutions of each complex were prepared in sterile nutrient broth to achieve concentrations ranging from 50 µg/mL to lower concentrations. Each test tube was inoculated with a bacterial suspension (approximately 1×10^6 CFU/mL) and incubated at 37°C for 24 hours. The MIC was determined as the lowest concentration of the complex that completely inhibited visible bacterial growth, observed as the absence of turbidity. To determine the MBC, 100 µL from each tube showing no visible growth was subcultured onto nutrient agar plates and incubated at 37°C for 24 hours. The MBC was defined as the lowest concentration of the complex where no bacterial growth appeared on the agar plate.

Antibiofilm Activity

The antibiofilm activity of the synthesized SMX-metal complexes was evaluated using the crystal violet tube method following I. Liaqat et al. (2023). A series of concentrations (5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 µg/mL) of each complex were prepared in sterile nutrient broth. Each concentration was inoculated with 100 µL of bacterial suspension (*E. coli*, *P. aeruginosa*, and *B. licheniformis*) to achieve a final bacterial concentration of approximately 1×10^6 CFU/mL. The tubes were incubated at 37°C for 24 hours to allow biofilm formation. After incubation, the planktonic bacteria were removed by gently washing the tubes with sterile saline. The biofilm was stained by adding 1 mL of 0.1% crystal violet solution and incubating the tubes for 15 minutes at room temperature. The excess stain was washed away, and the biofilm-bound crystal violet was solubilized by adding 1 mL of 95% ethanol. The absorbance was measured at 590 nm using a spectrophotometer. The antibiofilm activity was determined by comparing the optical density (OD) at 590 nm of the treated samples to the untreated control. A decrease in absorbance indicates a reduction in biofilm formation, and the concentration required to inhibit biofilm formation was identified.

Statistical Analysis

All data were analyzed using Statistical Package for the Social Sciences (SPSS, version 26). The antimicrobial, antibiofilm, MIC, and MBC values were subjected to one-way analysis of variance (ANOVA) followed by post hoc tests to assess significant differences between groups. Results were considered statistically significant at $p < 0.05$. Graphs illustrating antimicrobial activity and other results were generated using GraphPad Prism 10. FTIR spectra were processed and plotted using Origin software. All values are presented as mean $\pm$ standard error of mean

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(SEM) from triplicate experiments.

Results

The FTIR spectra of the synthesized SMX-metal complexes (SMX_Cu, SMX_Co, SMX_Cd, and SMX_Mn) were recorded to analyze the coordination of metal ions with the SMX ligand (Figure 1). The SMX_Cu complex showed key peaks at 479 cm^{-1} , 811 cm^{-1} , and 1423 cm^{-1} , which are attributed to the metal-ligand interaction and the characteristic vibrations of the SMX functional groups. The SMX_Co complex exhibited peaks at 515 cm^{-1} , 752 cm^{-1} , 1392 cm^{-1} , and 2226 cm^{-1} , indicating the coordination of cobalt with SMX. The SMX_Cd complex showed prominent peaks at 404 cm^{-1} , 713 cm^{-1} , 1383 cm^{-1} , 1963 cm^{-1} , 2531 cm^{-1} , and 3184 cm^{-1} , confirming the presence of cadmium coordination. The SMX_Mn complex displayed peaks at 493 cm^{-1} , 769 cm^{-1} , 1407 cm^{-1} , 2223 cm^{-1} , and 3167 cm^{-1} , suggesting manganese coordination. These shifts in the spectra, compared to the free SMX spectrum, confirm that the metal ions successfully coordinated with the sulfonamide group of SMX.

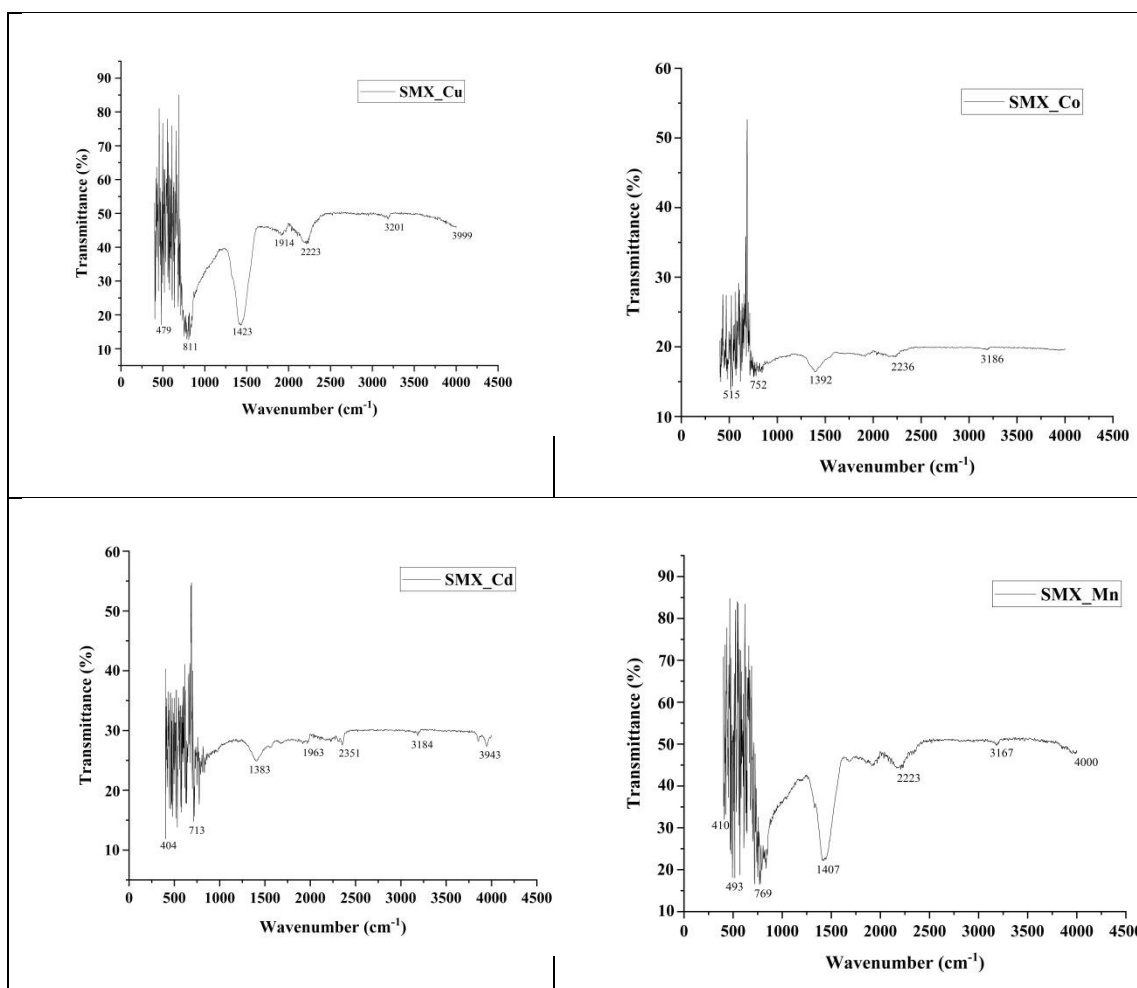
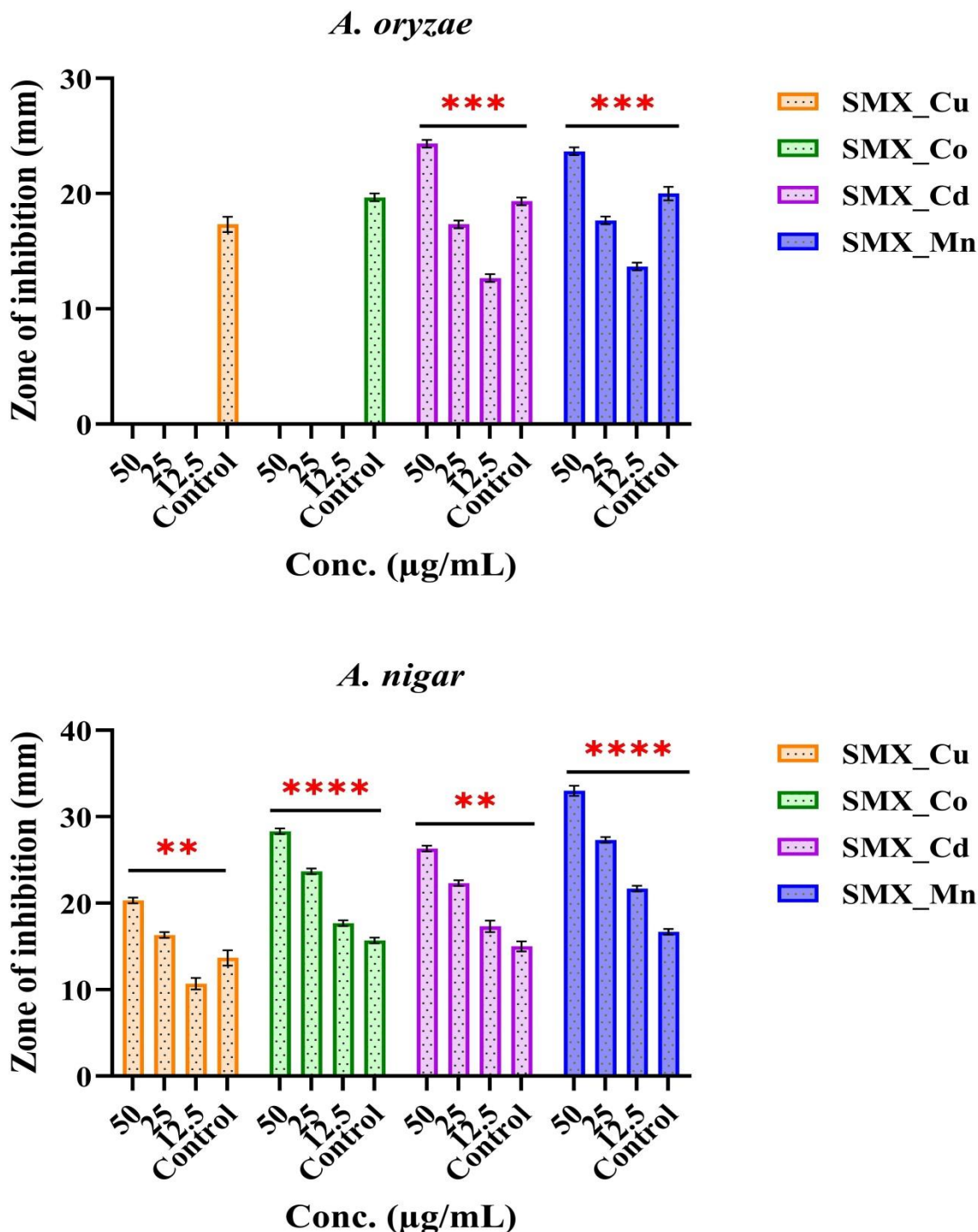


Figure 1. FTIR Spectra of SMX-Metal Complexes. FTIR spectra of sulfamethoxazole (SMX) complexes with copper (SMX_Cu), cobalt (SMX_Co), cadmium (SMX_Cd),

and manganese (SMX_Mn) showing characteristic transmittance peaks in the range of 4000 to 400 cm^{-1} . The spectra demonstrate the characteristic functional group vibrations of SMX and the shifts due to the coordination of metal ions with the sulfonamide group of SMX. Notable peaks corresponding to the metal-ligand interactions can be seen at various wavenumbers for each complex.

Antifungal activity

For *A. oryzae*, at a concentration of 50 $\mu\text{g/mL}$, none of the complexes (SMX_Cu, SMX_Co, SMX_Mn) exhibited significant activity, with a zone of inhibition (ZOI) of 0.0 ± 0.0 mm. However, the SMX_Cd complex showed a ZOI of 24.3 ± 0.3 mm at the same concentration. At 25 $\mu\text{g/mL}$, the SMX_Cd complex exhibited a ZOI of 17.3 ± 0.3 mm, while SMX_Mn showed a ZOI of 17.6 ± 0.3 mm. At the lowest concentration of 12.5 $\mu\text{g/mL}$, SMX_Cd showed a ZOI of 12.6 ± 0.3 mm, and SMX_Mn showed 13.6 ± 0.3 mm. For *A. niger*, at 50 $\mu\text{g/mL}$, SMX_Mn exhibited the largest ZOI of 33.0 ± 0.5 mm, followed by SMX_Co (28.3 ± 0.3 mm), SMX_Cd (26.3 ± 0.3 mm), and SMX_Cu (20.3 ± 0.3 mm). At 25 $\mu\text{g/mL}$, SMX_Mn showed the greatest ZOI (27.3 ± 0.3 mm), followed by SMX_Co (23.6 ± 0.3 mm), SMX_Cd (22.3 ± 0.3 mm), and SMX_Cu (16.3 ± 0.3 mm). At 12.5 $\mu\text{g/mL}$, SMX_Mn showed a ZOI of 21.6 ± 0.3 mm, while SMX_Co, SMX_Cd, and SMX_Cu showed ZOIs of 17.6 ± 0.3 mm, 17.3 ± 0.6 mm, and 10.6 ± 0.6 mm, respectively. The control (voriconazole at 50 $\mu\text{g/mL}$) showed a ZOI of 17.3 ± 0.6 mm for *A. oryzae* and 13.6 ± 0.8 mm for *A. niger*. Overall, SMX-metal complexes, especially SMX_Cd and SMX_Mn, demonstrated promising antifungal activity, particularly against *A. niger*, with SMX_Mn showing the strongest activity across the concentrations tested (Figure 2A-B). The one way ANOVA followed by posthoc Tukey test showed the results were highly significant ($P \leq 0.001$)



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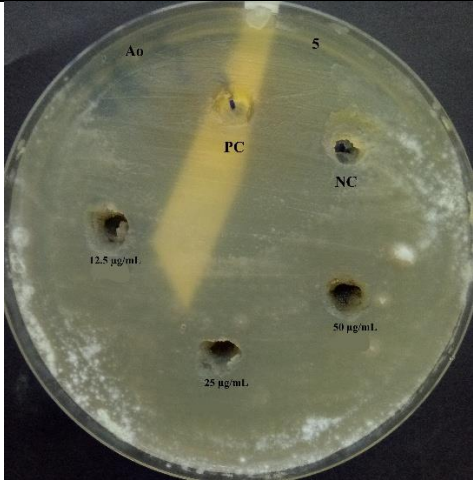
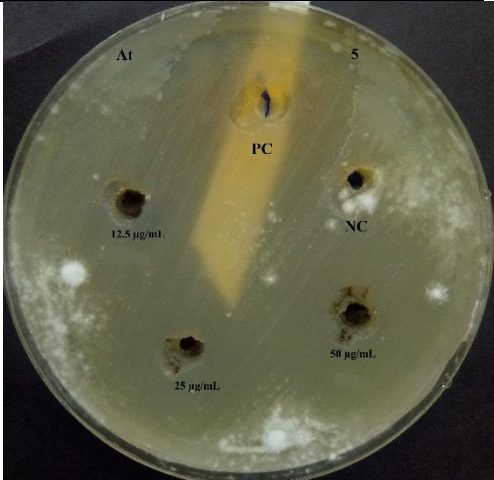
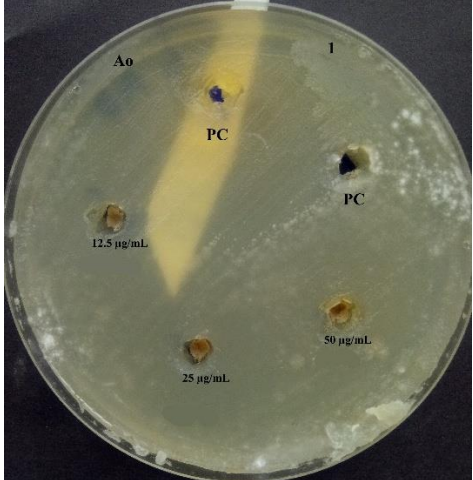
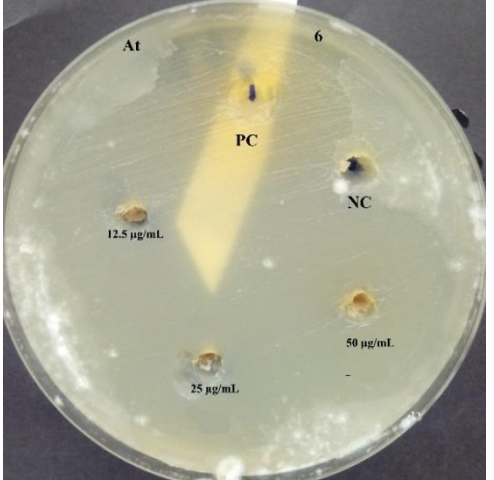
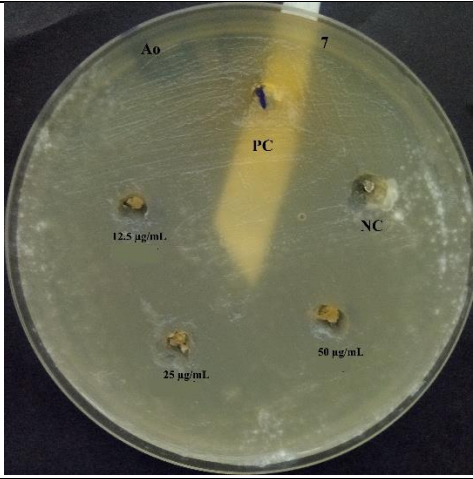
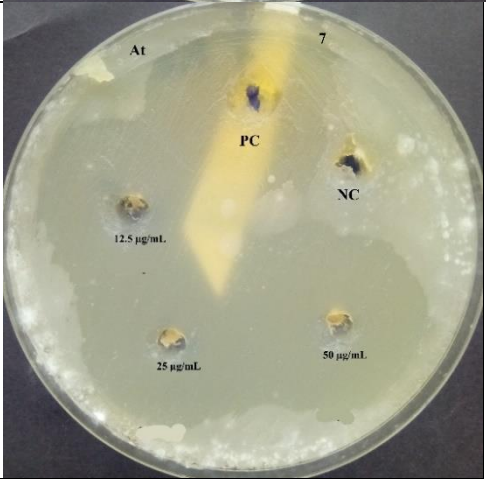
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concentrations (50, 25, and 12.5 $\mu\text{g/mL}$). The data are shown as mean $\pm$ standard deviation, with significant differences between groups indicated by asterisks ($p < 0.01$, $*p < 0.001$). SMX_Cd and SMX_Mn complexes exhibit the strongest antifungal activity, particularly at higher concentrations.

Complexes	<i>A. oryzae</i>	<i>A. niger</i>
SMX-Cu		
SMX-Co		
SMX-Cd		

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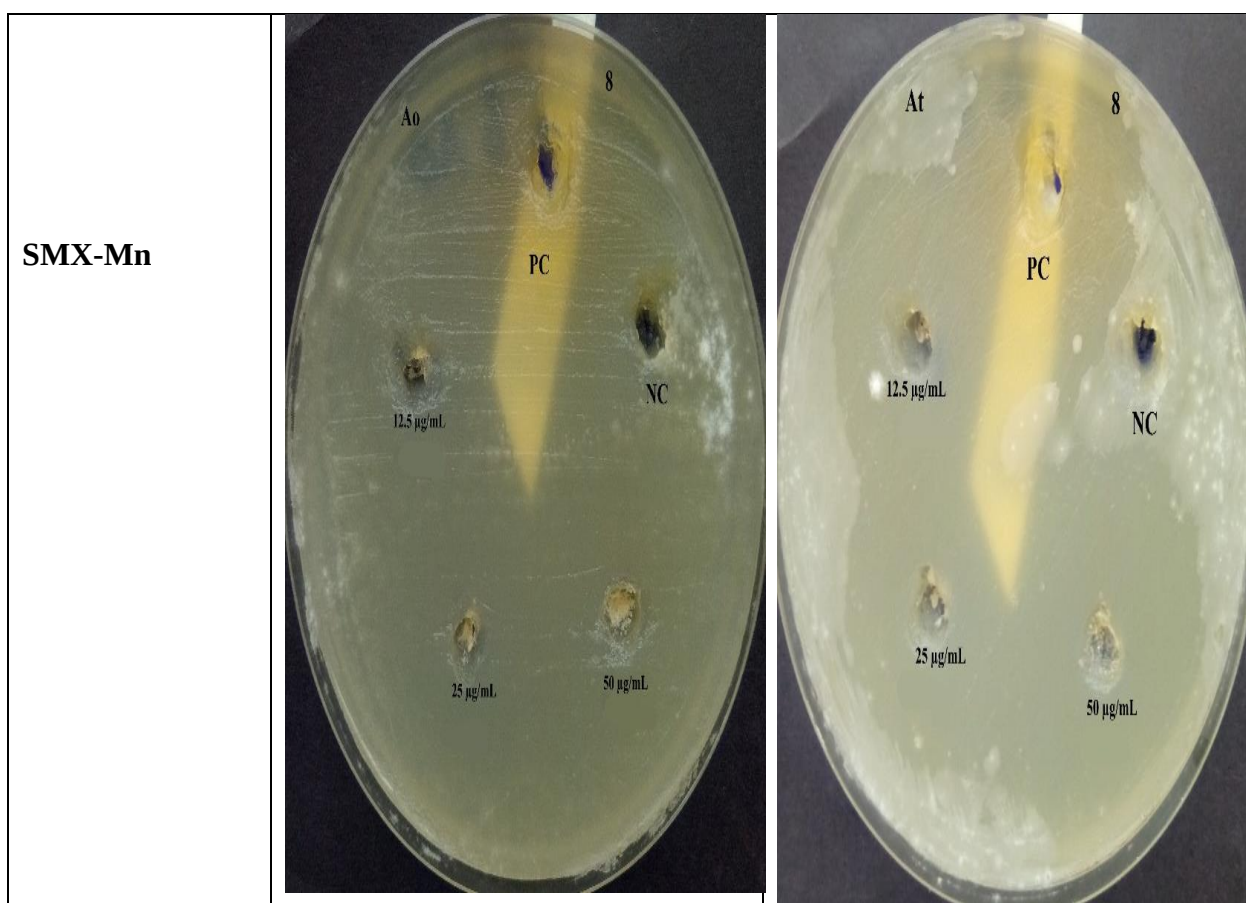
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Figure 2B. Antifungal Activity of SMX-Metal Complexes Against *A. oryzae* and *A. niger*

Minimum Inhibitory Concentration and Minimum Fungicidal Concentration

The MIC and MFC of the SMX-metal complexes against *A. niger* and *A. oryzae* were determined using the tube dilution method. The results are presented in Table 1. For *A. niger*, the MIC values for the complexes were as follows: SMX_Cu (5.3 ± 0.3 µg/mL), SMX_Co (2.6 ± 0.1 µg/mL), SMX_Cd (2.6 ± 0.1 µg/mL), and SMX_Mn (2.6 ± 0.1 µg/mL). The MFC values for *A. niger* were 10.0 ± 0.5 µg/mL for SMX_Cu, 5.3 ± 0.3 µg/mL for SMX_Co, 5.6 ± 0.6 µg/mL for SMX_Cd, and 5.3 ± 0.3 µg/mL for SMX_Mn. For *A. oryzae*, the MIC values were: SMX_Cu (48.3 ± 1.6 µg/mL), SMX_Co (53.3 ± 1.6 µg/mL), SMX_Cd (5.3 ± 0.3 µg/mL), and SMX_Mn (2.6 ± 0.3 µg/mL). The MFC values for *A. oryzae* were 56.6 ± 3.3 µg/mL for SMX_Cu, 0.0 ± 0.0 µg/mL for SMX_Co, 10.6 ± 0.6 µg/mL for SMX_Cd, and 10.6 ± 0.6 µg/mL for SMX_Mn.

Table 1. Minimum inhibitory concentration and minimum fungicidal concentration

Sr. No.	Compounds	Fungal Strains	MIC	MFC
1	SMX_Cu	A. nigar	5.3 ± 0.3	10.0 ± 0.5
		A. oryzae	48.3 ± 1.6	56.6 ± 3.3
2	SMX_Co	A. nigar	2.6 ± 0.1	5.3 ± 0.3
		A. oryzae	53.3 ± 1.6	0.0 ± 0.0
3	SMX_Cd	A. nigar	2.6 ± 0.1	5.6 ± 0.6
		A. oryzae	5.3 ± 0.3	10.6 ± 0.6
4	SMX_Mn	A. nigar	2.6 ± 0.1	5.3 ± 0.3
		A. oryzae	2.6 ± 0.3	10.6 ± 0.6

Antibacterial activity

Against *E. coli*, at a concentration of 50 µg/mL, the SMX_Cu complex showed a ZOI of 3.3 ± 0.3 mm, SMX_Co showed 6.0 ± 0.0 mm, SMX_Cd showed 12.3 ± 0.3 mm, and SMX_Mn exhibited the largest ZOI of 12.6 ± 0.3 mm. At 25 µg/mL, SMX_Cd and SMX_Mn displayed ZOIs of 10.3 ± 0.3 mm and 8.0 ± 0.0 mm, respectively, while SMX_Cu and SMX_Co showed no activity. At 12.5 µg/mL, SMX_Cd and SMX_Mn exhibited ZOIs of 3.3 ± 0.3 mm and 4.3 ± 0.3 mm, respectively. The control showed ZOIs of 6.3 ± 0.3 mm for SMX_Cu, 7.3 ± 0.3 mm for SMX_Co, 6.3 ± 0.3 mm for SMX_Cd, and 6.0 ± 0.0 mm for SMX_Mn. For *P. aeruginosa*, at 50 µg/mL, SMX_Mn showed the largest ZOI of 14.3 ± 0.3 mm, followed by SMX_Cd (13.3 ± 0.5 mm) and SMX_Co (12.3 ± 0.3 mm). The SMX_Cu complex showed no activity. At 25 µg/mL, SMX_Mn and SMX_Co displayed ZOIs of 10.3 ± 0.3 mm and 7.6 ± 0.4 mm, respectively. SMX_Cd showed a ZOI of 5.6 ± 0.5 mm. At 12.5 µg/mL, SMX_Mn, SMX_Co, and SMX_Cd showed ZOIs of 3.3 ± 0.3 mm, 4.3 ± 0.3 mm, and 3.6 ± 0.4 mm, respectively. The control showed ZOIs of 5.6 ± 0.3 mm for SMX_Cu, 8.3 ± 0.3 mm for SMX_Co, 7.6 ± 0.5 mm for SMX_Cd, and 3.6 ± 0.3 mm for SMX_Mn. For *B. licheniformis*, at 50 µg/mL, SMX_Mn showed the largest ZOI of 15.6 ± 0.6 mm, followed by SMX_Cd (12.3 ± 0.3 mm), SMX_Co (10.3 ± 0.4 mm), and SMX_Cu (8.3 ± 0.3 mm). At 25 µg/mL, SMX_Mn and SMX_Cd displayed ZOIs of 8.6 ± 0.4 mm, while SMX_Co and SMX_Cu showed ZOIs of 8.3 ± 0.4 mm and 6.3 ± 0.4 mm, respectively. At 12.5 µg/mL, SMX_Mn, SMX_Co, and SMX_Cu exhibited ZOIs of 6.3 ± 0.5 mm, 6.3 ± 0.4 mm, and 5.3 ± 0.3 mm, respectively. The control showed ZOIs of 8.6 ± 0.3 mm for SMX_Cu, 8.6 ± 0.4 mm for SMX_Co, 8.6 ± 0.3 mm for SMX_Cd, and 11.6 ± 0.3 mm for SMX_Mn (Figure 3A-B).

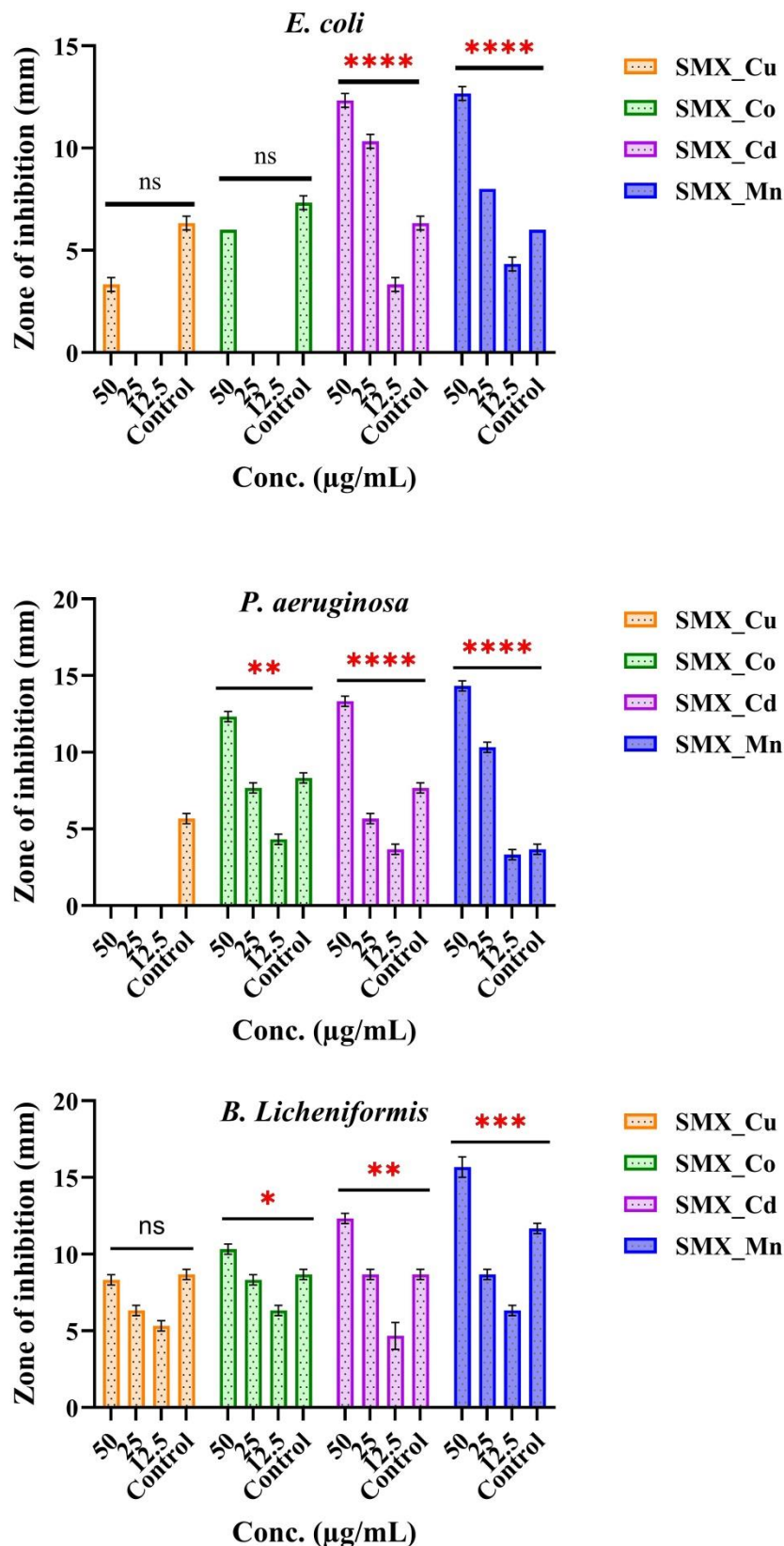
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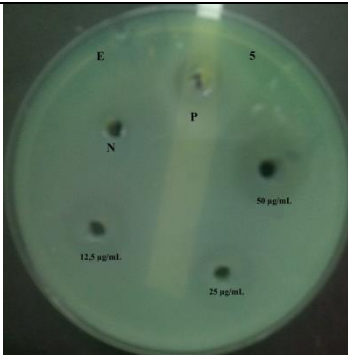
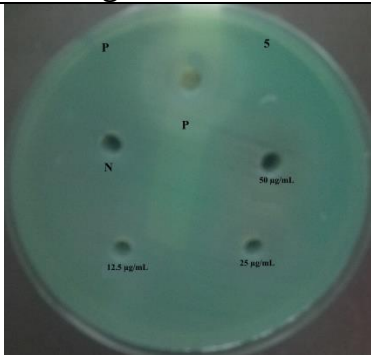
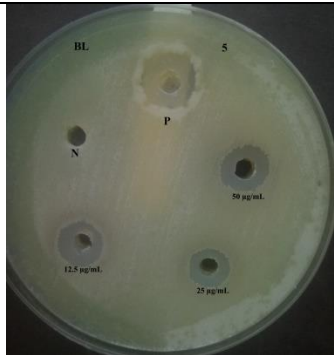
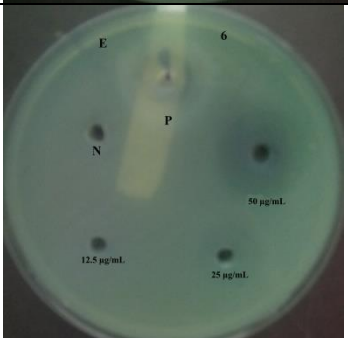
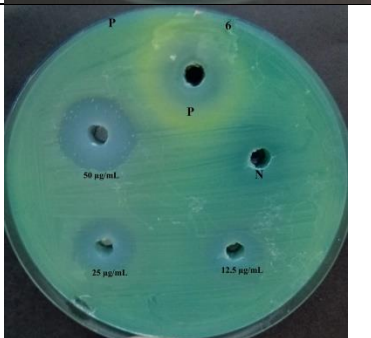
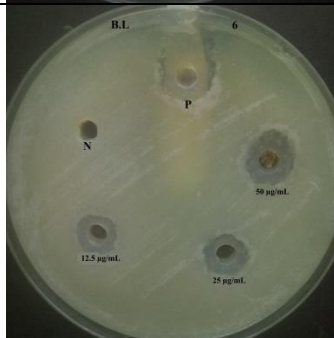
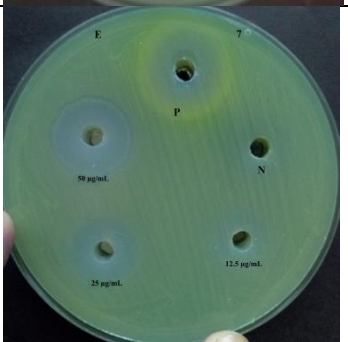
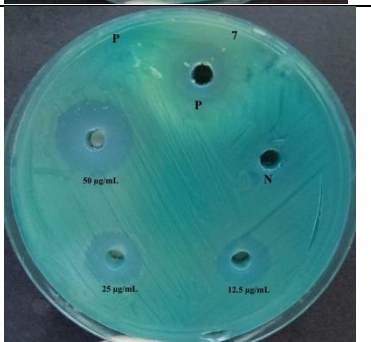
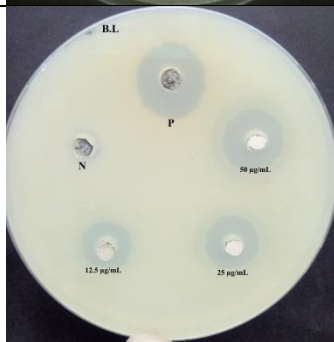
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Figure 3A. Antibacterial Activity of SMX-Metal Complexes Against *E. coli*, *P. aeruginosa*, and *B. licheniformis*. Bar graphs representing the zone of inhibition (ZOI) (mm) for the antibacterial activity of SMX-metal complexes (SMX_Cu, SMX_Co, SMX_Cd, and SMX_Mn) against *Escherichia coli* (top), *Pseudomonas aeruginosa* (middle), and *Bacillus licheniformis* (bottom) at various concentrations (50, 25, and 12.5 µg/mL). The data are shown as mean ± standard deviation, with significant differences between groups indicated by asterisks ($p < 0.05$, $*p < 0.01$, $**p < 0.001$, ns = not significant). SMX_Mn and SMX_Cd complexes exhibit the strongest antibacterial activity, particularly at higher concentrations.

Complexes	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. licheniformis</i>
SMX-Cu			
SMX-Co			
SMX-Cd			

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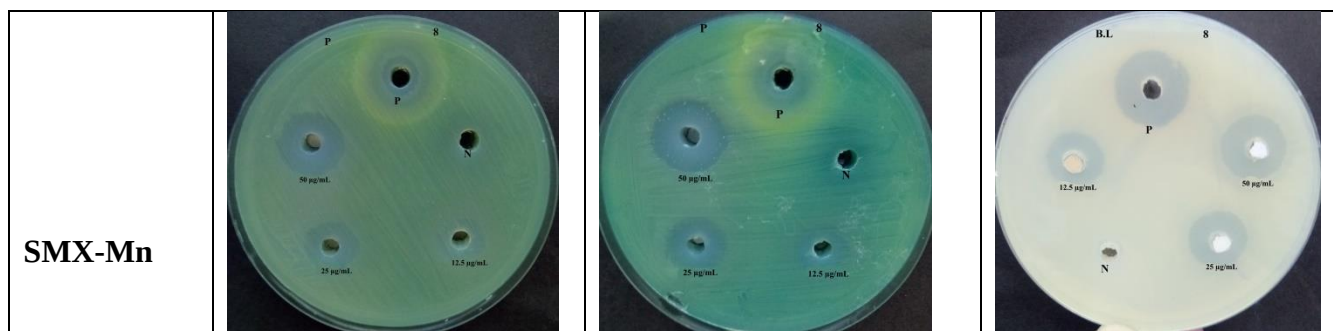
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Figure 3B. Antibacterial Activity of SMX-Metal Complexes Against *E. coli*, *P. aeruginosa*, and *B. licheniformis*.

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The MIC and MBC of the SMX-metal complexes against *E. coli*, *B. licheniformis*, and *P. aeruginosa* are summarized in Table 2. For *E. coli*, the MIC values were as follows: SMX_Cu ($53.3 \pm 1.6 \mu\text{g/mL}$), SMX_Co ($43.3 \pm 1.6 \mu\text{g/mL}$), SMX_Cd ($9.3 \pm 0.6 \mu\text{g/mL}$), and SMX_Mn ($9.6 \pm 0.3 \mu\text{g/mL}$). The corresponding MBC values were: SMX_Cu ($0.0 \pm 0.0 \mu\text{g/mL}$), SMX_Co ($49.3 \pm 0.6 \mu\text{g/mL}$), SMX_Cd ($21.0 \pm 0.5 \mu\text{g/mL}$), and SMX_Mn ($13.3 \pm 1.2 \mu\text{g/mL}$). For *B. licheniformis*, the MIC values were: SMX_Cu ($14.3 \pm 1.2 \mu\text{g/mL}$), SMX_Co ($10.0 \pm 0.5 \mu\text{g/mL}$), SMX_Cd ($8.6 \pm 0.8 \mu\text{g/mL}$), and SMX_Mn ($4.6 \pm 0.3 \mu\text{g/mL}$). The corresponding MBC values were: SMX_Cu ($29.6 \pm 0.8 \mu\text{g/mL}$), SMX_Co ($14.6 \pm 0.3 \mu\text{g/mL}$), SMX_Cd ($14.3 \pm 0.6 \mu\text{g/mL}$), and SMX_Mn ($7.6 \pm 0.3 \mu\text{g/mL}$). For *P. aeruginosa*, the MIC values were: SMX_Cu ($0.0 \pm 0.0 \mu\text{g/mL}$), SMX_Co ($14.6 \pm 0.8 \mu\text{g/mL}$), SMX_Cd ($11.6 \pm 0.3 \mu\text{g/mL}$), and SMX_Mn ($8.3 \pm 0.3 \mu\text{g/mL}$). The corresponding MBC values were: SMX_Cu ($0.0 \pm 0.0 \mu\text{g/mL}$), SMX_Co ($23.6 \pm 1.3 \mu\text{g/mL}$), SMX_Cd ($18.6 \pm 0.6 \mu\text{g/mL}$), and SMX_Mn ($14.0 \pm 1.0 \mu\text{g/mL}$).

Table 2. Minimum inhibitory concentration and minimum bactericidal concentration

Sr. No.	Compounds	Bacterial Strains	MIC	MBC
1	SMX_Cu	<i>E. coli</i>	53.3 ± 1.6	0.0 ± 0.0
		<i>B. licheniformis</i>	14.3 ± 1.2	29.6 ± 0.8
		<i>P. aeruginosa</i>	0.0 ± 0.0	0.0 ± 0.0
2	SMX_Co	<i>E. coli</i>	43.3 ± 1.6	49.3 ± 0.6
		<i>B. licheniformis</i>	10.0 ± 0.5	14.6 ± 0.3
		<i>P. aeruginosa</i>	14.6 ± 0.8	23.6 ± 1.3
3	SMX_Cd	<i>E. coli</i>	9.3 ± 0.6	21.0 ± 0.5
		<i>B. licheniformis</i>	8.6 ± 0.8	14.3 ± 0.6
		<i>P. aeruginosa</i>	11.6 ± 0.3	18.6 ± 0.6
4	SMX_Mn	<i>E. coli</i>	9.6 ± 0.3	13.3 ± 1.2
		<i>B. licheniformis</i>	4.6 ± 0.3	7.6 ± 0.3
		<i>P. aeruginosa</i>	8.3 ± 0.3	14.0 ± 1.0

Antibiofilm Potential

For the SMX_Co complex, at a concentration of 50 µg/mL, the antibiofilm inhibition was $35.0 \pm 0.3\%$ for *E. coli*, $100.0 \pm 0.0\%$ for *B. licheniformis*, and $100.0 \pm 0.0\%$ for *P. aeruginosa*. At 40 µg/mL, the complex showed $9.0 \pm 0.4\%$ inhibition against *E. coli*, $100.0 \pm 0.0\%$ against *B. licheniformis*, and $88.0 \pm 0.6\%$ against *P. aeruginosa*. For the SMX_Cd complex, at 50 µg/mL, the antibiofilm inhibition was $100.0 \pm 0.0\%$ for *E. coli*, *B. licheniformis*, and *P. aeruginosa*. At 40 µg/mL, the inhibition was $97.3 \pm 0.3\%$ for *E. coli*, $95.6 \pm 0.3\%$ for *B. licheniformis*, and $100.0 \pm 0.0\%$ for *P. aeruginosa*. For the SMX_Mn complex, at 50 µg/mL, the antibiofilm inhibition was $100.0 \pm 0.0\%$ for *E. coli*, *B. licheniformis*, and *P. aeruginosa*. At 40 µg/mL, the inhibition was $86.3 \pm 0.6\%$ for *E. coli*, $100.0 \pm 0.0\%$ for *B. licheniformis*, and $87.3 \pm 0.5\%$ for *P. aeruginosa* (Figure 4).

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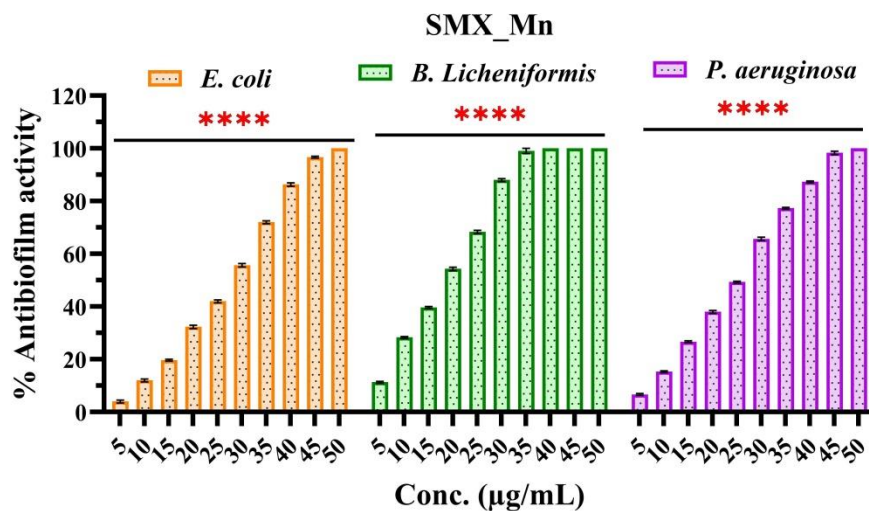
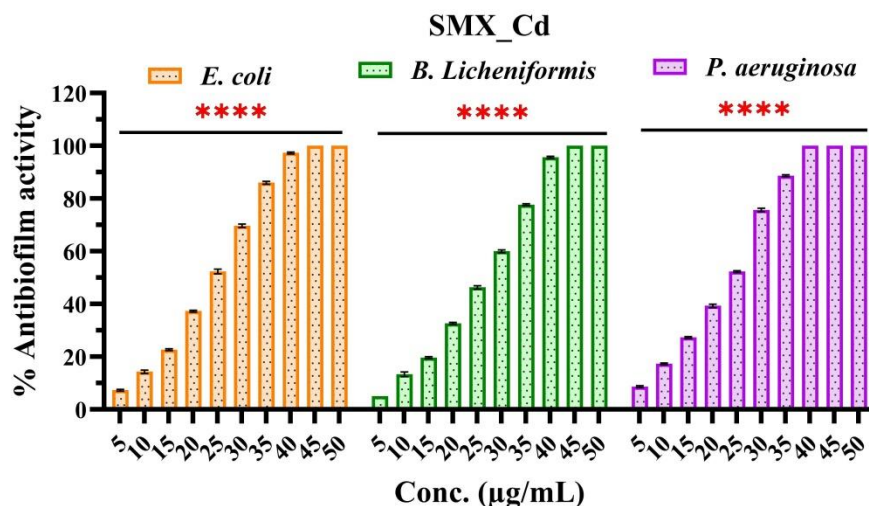
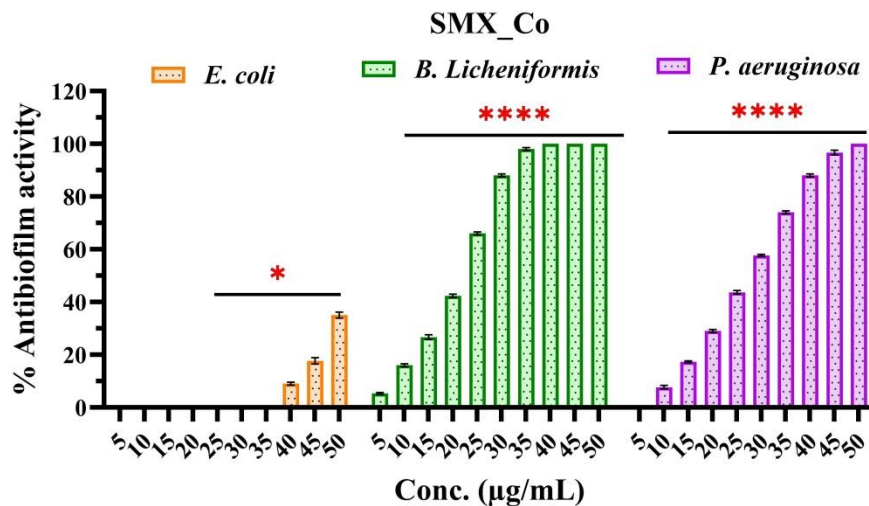


Figure 4. Antibiofilm Activity of SMX-Metal Complexes Against *E. coli*, *B. licheniformis*, and *P. aeruginosa* Bar graphs showing the percentage of antibiofilm activity of SMX-metal complexes (SMX_Co, SMX_Cd, and SMX_Mn) against *Escherichia coli* (left), *Bacillus licheniformis* (middle), and *Pseudomonas aeruginosa* (right) at various concentrations (5 to 50 µg/mL). The data are shown as mean ± standard deviation, with significant differences indicated by asterisks ($p < 0.05$, * $p < 0.01$, ** $p < 0.001$). The complexes demonstrated significant antibiofilm activity, particularly at higher concentrations, with SMX_Mn and SMX_Cd showing the most effective results.

Discussion

The FTIR spectra confirm the coordination of copper, cobalt, cadmium, and manganese ions with the sulfamethoxazole ligand. In each case, significant shifts in the characteristic peaks of SMX were observed, particularly in the regions corresponding to the sulfonamide and sulfonate groups. For example, the shift in the peak at 479 cm^{-1} in SMX_Cu and similar shifts in SMX_Co, SMX_Cd, and SMX_Mn complexes suggest that the metal ions interact with the sulfonamide nitrogen and oxygen atoms, leading to changes in the electronic environment of the functional groups (Nassar et al., 2023). These shifts indicate the successful formation of metal-ligand bonds, which is consistent with findings from previous studies on metal complexes of sulfonamides (Mishra et al., 2025). The appearance of new peaks, especially at lower wavenumbers, further supports the formation of stable complexes. The FTIR analysis, therefore, provides crucial evidence of the interaction between SMX and the metal ions, confirming the formation of SMX-metal complexes that are expected to enhance the antimicrobial activity of the ligand (Abbas et al., 2025).

The antifungal activity of the SMX-metal complexes against *A. oryzae* and *A. niger* showed promising results, particularly with the SMX_Cd and SMX_Mn complexes. SMX_Cd exhibited moderate activity against *A. oryzae* at higher concentrations, while SMX_Mn demonstrated the strongest activity against *A. niger* across all concentrations tested, particularly at 50 µg/mL. The metal ions likely enhance the activity of SMX by altering its binding properties and increasing membrane permeability, a mechanism reported for metal-antibiotic complexes (Hashem et al., 2023). These results align with studies showing that metal complexes can improve the solubility and bioavailability of sulfonamides, making them more effective against resistant fungal strains (Wu et al., 2025). The lack of activity at higher concentrations of SMX_Cu and SMX_Co against *A. oryzae* suggests that certain metal ions may not effectively enhance antifungal properties for all strains, which is consistent with previous findings that metal ion type can significantly influence antimicrobial efficacy (Slavin & Bach, 2022).

The low MIC and MFC values for SMX_Mn and SMX_Co against *A. niger* suggest that these complexes may offer more effective treatments for this fungal strain, potentially due to enhanced metal-ligand interaction, which is known to increase the antimicrobial properties of sulfonamide complexes (Frei et al., 2022). These results highlight the potential of SMX-metal complexes as promising candidates for the development of new antifungal agents.

The results of the antibacterial activity demonstrate that the SMX-metal complexes

exhibit varying degrees of effectiveness against the bacterial strains tested. The SMX_Mn complex showed the most potent antibacterial activity against all three bacterial strains. At 50 µg/mL, it produced the largest zone of inhibition (ZOI) for *E. coli*, *P. aeruginosa*, and *B. licheniformis*, indicating strong antibacterial properties. This suggests that the manganese ion plays a significant role in enhancing the antimicrobial activity of SMX, potentially due to its ability to alter the drug's electronic properties and enhance its penetration into bacterial cells, as seen in similar studies of metal-based antibiotics (Friães et al., 2023).

The antibacterial findings highlight the importance of the metal ion in modulating the antibacterial activity of SMX. The presence of certain metal ions, such as manganese and cadmium, appears to enhance the antimicrobial properties of SMX, possibly through interactions that increase the stability, solubility, and permeability of the complex, thus facilitating better bacterial inhibition (Godoy-Gallardo et al., 2021). In contrast, SMX_Cu showed limited efficacy, suggesting that copper may not enhance SMX activity in the same way as other metal ions, a phenomenon that requires further investigation.

The MIC and MBC results demonstrate the varying effectiveness of the SMX-metal complexes against different bacterial strains. For *E. coli*, SMX_Cu exhibited the highest MIC (53.3 ± 1.6 µg/mL), but its MBC was reported as 0.0 ± 0.0 µg/mL, which suggests no significant bactericidal effect. This could indicate that while SMX_Cu inhibits bacterial growth, it does not effectively kill the bacteria, aligning with findings that copper-based complexes may act primarily as bacteriostatic agents (Serafin et al., 2023). In contrast, SMX_Co and SMX_Cd exhibited moderate bactericidal effects with MBC values of 49.3 ± 0.6 µg/mL and 21.0 ± 0.5 µg/mL against *E. coli*, respectively. SMX_Mn, with an MIC of 9.6 ± 0.3 µg/mL and MBC of 13.3 ± 1.2 µg/mL, demonstrated notable antibacterial activity, suggesting that manganese may play a significant role in enhancing the antimicrobial efficacy of SMX.

For *P. aeruginosa*, the SMX_Cu complex showed no antibacterial activity (MIC = 0.0 ± 0.0 µg/mL, MBC = 0.0 ± 0.0 µg/mL), while SMX_Mn exhibited the lowest MIC (8.3 ± 0.3 µg/mL) and MBC (14.0 ± 1.0 µg/mL), suggesting it is the most effective against *P. aeruginosa*. These results suggest that the metal ion plays a crucial role in modulating the antimicrobial activity of the sulfonamide, with manganese and cadmium complexes showing the strongest activity against the tested bacterial strains. These findings underscore the potential of SMX-metal complexes as effective antibacterial agents, with manganese and cadmium complexes offering the best balance of bacteriostatic and bactericidal properties (Rozbicki & Branowska, 2025).

The results show that the SMX-metal complexes effectively inhibited biofilm formation by *E. coli*, *B. licheniformis*, and *P. aeruginosa* in a concentration-dependent manner. SMX_Cd and SMX_Mn complexes exhibited the strongest antibiofilm activity across all concentrations, with 100% inhibition at 50 µg/mL for all three bacterial strains, particularly against *B. licheniformis* and *P. aeruginosa*. These results suggest that the cadmium and manganese complexes may effectively disrupt biofilm formation by interfering with bacterial adhesion and biofilm matrix synthesis, likely due to enhanced metal-ligand interactions (Olar et al., 2022).

The SMX_Co complex also demonstrated significant biofilm inhibition, especially

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against *B. licheniformis* and *P. aeruginosa*, with 100% inhibition at 50 µg/mL. The ability of SMX_Co to inhibit biofilm formation could be attributed to cobalt's unique properties in affecting bacterial cellular structures and biofilm matrix components. However, the SMX_Co complex was less effective against *E. coli*, suggesting a strain-specific response to the metal ion coordination (Yadav et al., 2025).

The concentration-dependent inhibition observed in all three complexes indicates their potential for developing as effective antibiofilm agents. The 100% inhibition at 50 µg/mL demonstrates the potential of these complexes for combating biofilm-related infections, which are often resistant to conventional antibiotics due to the protective nature of the biofilm matrix (Nadar et al., 2022).

Conclusion

The results of this study demonstrate that SMX-metal complexes, particularly those with cadmium (SMX_Cd) and manganese (SMX_Mn), exhibit significant antimicrobial and antibiofilm activities against both fungal and bacterial pathogens. These complexes showed promising antifungal effects, particularly against *A. niger* and *A. oryzae*, with SMX_Mn and SMX_Cd complexes being the most effective. Additionally, the SMX-Mn complex demonstrated strong antibacterial activity against *E. coli*, *P. aeruginosa*, and *B. licheniformis*, with excellent antibiofilm activity observed at higher concentrations. The FTIR analysis confirmed successful metal-ligand coordination, which likely contributes to the enhanced antimicrobial properties of these complexes. The SMX-metal complexes, especially those involving manganese and cadmium, hold significant potential as novel antimicrobial and antibiofilm agents, offering a promising approach to combat antibiotic-resistant pathogens and biofilm-related infections. Further studies, including in vivo testing, are necessary to fully understand the therapeutic potential of these complexes.

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