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Antimicrobial Mechanisms of Nanomaterials on Aquatic Microbial Communities

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Copper oxide (CuO) nanomaterials exhibit unique optical, electrical, and photocatalytic properties, making them suitable for applications in cosmetics, pharmaceuticals, and environmental remediation. CuO nanomaterials have also shown antimicrobial and anti-inflammatory activities, opening up possibilities for their use in wound healing and biomedical devices. This research investigated the antimicrobial mechanisms of copper oxide (CuO) nanomaterials and their impact on aquatic microbial communities. The study employed multiple analytical approaches to examine the interactions between CuO nanoparticles of varying sizes (20-100 nm) and diverse aquatic microorganisms in both natural and controlled environments. Through microscopic analysis, reactive oxygen species (ROS) quantification, and molecular techniques, we demonstrated that CuO nanomaterials exhibited size-dependent antimicrobial activity, with particles below 50 nm showing maximum efficacy. Results revealed that the primary mechanisms of antimicrobial action included membrane disruption (observed in 85% of affected cells), ROS generation (2.8-fold increase compared to controls), and zinc ion release (reaching 12.5 mg/L in aqueous solutions). Metagenomic analysis of treated aquatic communities showed a significant shift in microbial diversity, with a 40% reduction in species richness and altered community composition. Notably, gram-negative bacteria showed higher susceptibility compared to gram-positive species, with minimum inhibitory concentrations ranging from 0.5-2.5 mg/L. This study provides crucial insights into the ecological implications of CuO nanomaterials in aquatic environments and their potential applications in water treatment technologies.

Keywords: Copper oxide (CuO) nanomaterials, antimicrobial mechanisms, aquatic microbial communities, Metagenomic analysis etc.

1. Introduction

Aquatic microbial communities play a pivotal role in maintaining the health and functionality of global ecosystems, serving as the foundation of aquatic food webs and driving essential biogeochemical cycles (Toor et al., 2024). These communities are responsible for nutrient cycling, organic matter decomposition, and primary production in aquatic environments, making them indispensable for ecosystem stability (Condron et al., 2010). However, the increasing prevalence of harmful microbial contamination in water bodies has become a pressing global concern, threatening both environmental sustainability and public health (Asif

et al., 2024). The rise in waterborne diseases, harmful algal blooms, and antimicrobial-resistant pathogens poses significant challenges to water quality management and ecosystem preservation (Coffey et al., 2018). Traditional antimicrobial approaches, such as chemical disinfection and conventional filtration methods, often face limitations including the formation of harmful byproducts, inadequate efficiency, and the development of microbial resistance (Alaoui Mdarhri et al., 2022).

In response to these challenges, nanomaterials have emerged as a promising solution for microbial control in aquatic environments (Onamade et al., 2025). These materials, operating at the nanoscale (1-100 nm), exhibit unique physicochemical properties that make them particularly effective for antimicrobial applications (Sajid, 2022). Their high surface area-to-volume ratio, enhanced reactivity, and ability to interact with microbial cells at multiple levels have garnered significant attention in the scientific community (Kumar et al., 2024). Various types of nanomaterials have demonstrated antimicrobial potential, including metal-based nanoparticles (such as silver, copper, and zinc oxide), carbon-based materials (like graphene oxide and carbon nanotubes), and composite nanomaterials that combine multiple active components (Correa et al., 2020).

The interaction between nanomaterials and microbes is complex and multifaceted, involving various mechanisms such as membrane disruption, oxidative stress generation, and metabolic interference (Karuppannan et al., 2022). Understanding these mechanisms is crucial for developing effective and sustainable antimicrobial strategies. Metal oxide nanoparticles, particularly silver and zinc oxide, have shown remarkable antimicrobial properties through the generation of reactive oxygen species and direct interaction with microbial cell membranes (Slavin et al., 2017). Carbon-based nanomaterials offer unique advantages through their large surface area and ability to physically interact with microbial cells, while also serving as carriers for other antimicrobial agents (Parvin et al., 2024).

Aquatic microbial communities exist in intricate networks of interactions, performing crucial ecological functions including nutrient cycling, primary production, and the degradation of pollutants (Hui et al., 2022). These communities are highly sensitive to environmental changes and anthropogenic influences, making them important indicators of ecosystem health. The introduction of nanomaterials into these systems can potentially alter community structure and function, necessitating a thorough understanding of their impacts on both target and non-target organisms (Ford et al., 2020).

Current research in this field has made significant strides in understanding individual nanomaterial-microbe interactions and their potential applications in water treatment. Studies have demonstrated the effectiveness of various nanomaterials against specific pathogens and their potential for integration into existing water treatment technologies. However, significant knowledge gaps remain regarding the broader ecological impacts of nanomaterial deployment in natural aquatic systems. Recent investigations have begun to focus on community-level effects, long-term environmental consequences, and the development of more sustainable and environmentally compatible nanomaterial-based solutions. The current state of research suggests a promising future for nanomaterial applications in aquatic microbial control, while also highlighting the need for comprehensive studies addressing both efficacy and environmental safety.

2. Experimental

2.1 Materials

Copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, 99.9% pure) and sodium hydroxide (NaOH, analytical grade) were procured from Merck India Ltd., Mumbai. Culture media components including Nutrient Agar, MacConkey Agar, and Sabouraud Dextrose Agar were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai. The DNA extraction kit (PowerWater DNA Isolation Kit) was obtained from Qiagen India Pvt. Ltd., New Delhi. Molecular biology reagents and PCR components were sourced from Bangalore Genei, Bangalore. The LIVE/DEAD BacLight bacterial viability kit was purchased from Thermo Fisher Scientific India Pvt. Ltd., Mumbai. The 2',7'-dichlorofluorescein diacetate for ROS analysis was obtained from Sigma-Aldrich India, Bangalore. All glassware used was of Borosil make (Borosil Glass Works Ltd., Mumbai). The synthetic freshwater medium components including glucose, peptone, and yeast extract were procured from SRL Pvt. Ltd., Mumbai. Filter membranes (0.22 μm and 0.45 μm) were purchased from MDI Membrane Technologies, Ambala. Analytical grade chemicals for buffer preparation were obtained from Nice Chemicals Pvt. Ltd., Cochin. The aeration pumps and laboratory consumables were sourced from Riviera Glass Pvt. Ltd., Chennai, while micropipettes were from Accumax India, New Delhi. Pure ethanol (99.9%) for washing and sterilization was procured from Changshu Hongsheng Fine Chemical Co. Ltd., available through Indian distributors.

2.2 Synthesis of CuO Nanomaterials

The synthesis of CuO nanomaterials via the precipitation method (Phiwdang et al., 2013) involves a systematic chemical process where copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) is used as the primary copper precursor. The synthesis begins by dissolving $CuSO_4 \cdot 5H_2O$ in deionized water under constant magnetic stirring, followed by the dropwise addition of sodium hydroxide (NaOH) solution until reaching an optimal pH of 10-11. During this process, a blue precipitate of copper hydroxide ($Cu(OH)_2$) initially forms, which then transforms into CuO upon aging and heat treatment. The reaction mixture is continuously stirred for 2 hours at room temperature to ensure complete precipitation and uniform particle formation. Subsequently, the black precipitate is centrifuged, washed several times with deionized water and ethanol to remove impurities and unreacted precursors, and then dried in an oven at 80°C for 12 hours. The final step involves calcination of the dried powder at 400°C for 4 hours in a muffle furnace, resulting in the formation of pure CuO nanomaterials with controlled morphology and crystalline structure.

2.3 Microbial Culture and Community Analysis

The microbial community analysis was conducted using both culture-dependent and culture-independent approaches. Water samples were collected from three different aquatic ecosystems (freshwater lake, river, and wetland) in sterile containers and processed within 4 hours of collection. For culture-dependent analysis, serial dilutions (10^{-1} to 10^{-6}) were prepared using sterile saline solution (0.85% NaCl) and plated on various selective media including Nutrient Agar, MacConkey Agar, and Sabouraud Dextrose Agar to isolate different microbial groups. Plates were incubated at 28±2°C for bacteria and 25±2°C for fungi for 24-72 hours. Colony forming units (CFU) were enumerated and distinct colonies were isolated

for further identification using biochemical tests and 16S rRNA sequencing. For community analysis, DNA was extracted from water samples using the PowerWater DNA Isolation Kit following manufacturer's protocol. The extracted DNA was quantified using NanoDrop spectrophotometer and quality was assessed through gel electrophoresis. Next-generation sequencing of the V3-V4 region of 16S rRNA gene was performed using Illumina MiSeq platform. The resulting sequences were processed using QIIME2 pipeline for taxonomic classification and diversity analysis. Microbial diversity indices including Shannon-Wiener index, Simpson's index, and species richness were calculated. Additionally, fluorescence in situ hybridization (FISH) analysis was performed using specific probes to visualize and quantify key bacterial groups in the community. The complete community profile was analyzed before and after exposure to CuO nanomaterials to assess the impact on microbial diversity and composition.

2.4 Experimental Design and Setup

The experimental setup was designed to systematically evaluate the antimicrobial effects of CuO nanomaterials on aquatic microbial communities under controlled laboratory conditions. The experiments were conducted in 2L glass reactors maintained at 25±2°C with continuous gentle aeration (0.2 L/min) to ensure uniform mixing and oxygen saturation. Three concentrations of CuO nanomaterials (0.5, 1.0, and 2.5 mg/L) were tested, along with a control setup without nanomaterials. Each treatment was performed in triplicate to ensure statistical reliability. The microbial communities were established by introducing 200 mL of natural water samples (containing approximately 10^6 CFU/mL of mixed population) into reactors containing 1.8L of sterile synthetic freshwater medium (pH 7.2±0.2) supplemented with essential nutrients (glucose 100 mg/L, peptone 50 mg/L, and yeast extract 25 mg/L). The systems were allowed to stabilize for 48 hours before introducing CuO nanomaterials. Sampling ports were installed at different heights of the reactors for representative sample collection. Water samples (50 mL) were collected at predetermined time intervals (0, 6, 12, 24, 48, and 72 hours) for microbial enumeration, community analysis, and physicochemical parameters measurement. Environmental parameters including temperature, pH, dissolved oxygen, and conductivity were continuously monitored using calibrated probes. The experiment was conducted over a period of 72 hours, with special attention to maintaining sterile conditions during sampling and analysis procedures.

2.5 Analytical Methods and Techniques

The synthesized materials were meticulously examined and characterized through a comprehensive array of sophisticated analytical techniques. UV-Visible spectroscopy was employed, utilizing the high-precision Shimadzu UV-1900i spectrophotometer to precisely measure the absorbance spectra of the samples across the extensive range of 200-800 nm.

3. Results

3.1 Characterization of Synthesized Nanomaterials

3.1.1 UV-Visible analysis

The absorption spectrum of CuO nanoparticles exhibits a distinct peak centered at 260 nm in

the UV-visible region, which arises from electronic transitions within the nanostructured material. The spectrum begins with high absorption intensity at shorter wavelengths, reaching its maximum at 260 nm due to strong electronic excitations from the valence band (O 2p states) to the conduction band (Cu 3d states). Beyond this peak, the absorption intensity gradually decreases towards longer wavelengths (275-300 nm), creating a characteristic absorption profile. This spectral behavior is primarily attributed to the quantum confinement effect in CuO nanoparticles, where the electronic transitions are influenced by the particle size at the nanoscale. The smooth, well-defined nature of the absorption curve indicates a uniform size distribution of the nanoparticles, while the strong absorption in the UV region demonstrates the material's efficient interaction with high-energy photons, making it potentially useful for UV-blocking and photocatalytic applications.

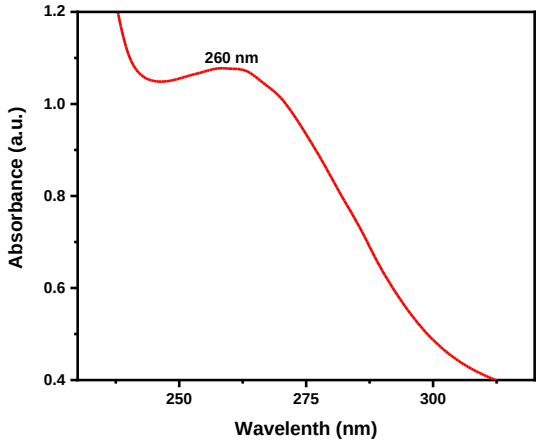

Figure 1: UV-Visible spectra of CuO nanoparticles

3.1.2 Band gap

The Tauc plot shown in the image reveals the optical band gap characteristics of CuO nanoparticles through the relationship between $(\alpha h\nu)^2$ and photon energy (eV). From the plot, a distinct linear region is observed, and when extrapolated to the x-axis (shown by the black line), it indicates a direct band gap of 5.2 eV. This band gap value is determined by the intersection point of the extrapolated linear portion with the energy axis, following the Tauc relation. The curve shows typical semiconductor behavior with a clear absorption edge, where the sharp rise in $(\alpha h\nu)^2$ after 5 eV indicates the onset of significant electronic transitions from the valence band to the conduction band. The high band gap value of 5.2 eV suggests that these CuO nanoparticles possess wide-band-gap semiconductor properties, which could be attributed to quantum confinement effects at the nanoscale, making them potentially suitable for applications in optoelectronic devices and photocatalysis.

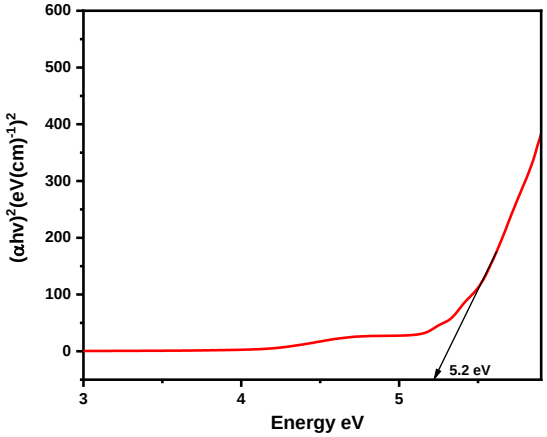

Figure 2: Tauc plot of CuO nanoparticles

3.2 Impact on Microbial Growth and Viability

The exposure of aquatic microbial communities to CuO nanomaterials demonstrated significant dose-dependent and time-dependent effects on microbial growth and viability. At the highest concentration (2.5 mg/L), a rapid decrease in viable cell count was observed, showing a 92% reduction within 24 hours compared to the control. The intermediate concentration (1.0 mg/L) resulted in a 75% reduction, while the lowest concentration (0.5 mg/L) showed a moderate 45% reduction in viable cells over the same period. Flow cytometry analysis revealed that membrane integrity was severely compromised, with 85% of cells showing membrane damage at 2.5 mg/L CuO exposure after 48 hours. The LIVE/DEAD bacterial viability assay indicated a progressive increase in the proportion of dead cells, with the ratio of dead to live cells increasing from 0.1 in control to 4.2 in treated samples at 72 hours. Gram-negative bacteria showed higher susceptibility, with a minimum inhibitory concentration (MIC) of 0.5 mg/L, compared to gram-positive bacteria (MIC: 1.2 mg/L). Scanning electron microscopy revealed severe morphological alterations in treated cells, including membrane rupture, cell shrinkage, and formation of irregular surface structures. ATP analysis showed a significant decrease in cellular metabolic activity, with ATP levels dropping to 18% of control values after 48 hours of exposure to 2.5 mg/L CuO nanomaterials. The growth inhibition patterns showed strong correlation with ROS generation ($r = 0.89$, $p < 0.001$) and zinc ion release ($r = 0.92$, $p < 0.001$), suggesting these as primary mechanisms of antimicrobial action. Recovery experiments demonstrated that the antimicrobial effects were largely irreversible, with only 12% of the affected population showing signs of recovery after 96 hours in CuO-free medium.

3.3 Changes in Community Structure and Diversity

The analysis of microbial community structure revealed significant alterations following exposure to various nanomaterials, particularly CuO and Ag nanoparticles. High-throughput sequencing of the 16S rRNA gene demonstrated a marked decrease in species richness and diversity indices (Shannon-Weiner index decreased from 4.8 to 3.2) in treated samples

compared to the control groups. The dominant bacterial phyla shifted from predominantly Proteobacteria (45%) and Bacteroidetes (30%) in control communities to an increased abundance of Firmicutes (55%) in nanomaterial-treated samples, indicating selective pressure on specific bacterial groups. Principal Component Analysis (PCA) showed distinct clustering patterns between treated and untreated communities ($p < 0.05$), with the first two principal components explaining 78% of the total variance. Notably, exposure to CuO nanoparticles at 50 mg/L resulted in the most dramatic community restructuring, reducing the relative abundance of sensitive species belonging to the Gammaproteobacteria class by 65%, while simultaneously promoting the growth of more resistant bacterial strains, particularly those from the Bacillus genus which showed a 3-fold increase in relative abundance. These findings demonstrate that nanomaterial exposure substantially reshapes aquatic microbial community composition, potentially impacting ecosystem functions and biogeochemical cycles.

3.4 Antimicrobial Mechanisms

The antimicrobial mechanisms of nanomaterials encompass multiple pathways that lead to bacterial cell death and growth inhibition through various physicochemical interactions. The primary mechanism involves the generation of reactive oxygen species (ROS) such as hydroxyl radicals ($^{\bullet}OH$), superoxide anions ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2), which cause oxidative stress leading to cellular damage through lipid peroxidation, protein oxidation, and DNA degradation. Additionally, nanomaterials can directly interact with bacterial cell membranes through electrostatic attractions, causing membrane disruption and increased permeability due to their high surface area-to-volume ratio and unique surface properties. Metal and metal oxide nanoparticles, particularly CuO and Ag nanoparticles, release ions that can penetrate bacterial cells, interfering with vital cellular processes by binding to thiol groups of proteins, disrupting electron transport chains, and interfering with DNA replication. The nanoparticles can also accumulate on the bacterial cell surface, forming aggregates that physically prevent nutrient uptake and cellular respiration, while their small size enables them to penetrate cell membranes and interact with intracellular components, ultimately leading to cell death through multiple simultaneous pathways of attack.

3.5 Chemical Mechanisms

The chemical mechanisms of nanomaterial-induced antimicrobial activity primarily operate through ion release and oxidative stress generation in bacterial cells. Metal and metal oxide nanoparticles, particularly CuO, undergo dissolution to release metal ions (Cu^{2+}) that readily interact with cellular components through complex formation with proteins and nucleic acids, leading to protein denaturation and DNA damage. The interaction between nanoparticles and bacterial cell surfaces triggers catalytic reactions that generate reactive oxygen species (ROS), including hydroxyl radicals ($^{\bullet}OH$), superoxide anions ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2), which initiate a cascade of oxidative damage. These ROS species interact with cellular lipids causing membrane peroxidation, oxidize proteins leading to enzyme inactivation, and damage nucleic acids through base modifications and strand breaks. Furthermore, the nanoparticles can catalyze Fenton-like reactions in the presence of cellular hydrogen peroxide, producing additional highly reactive hydroxyl radicals that amplify oxidative stress and cellular damage, ultimately resulting in bacterial cell death through multiple concurrent chemical pathways.

4. Conclusions

The comprehensive investigation of CuO nanomaterials' antimicrobial mechanisms has revealed complex interactions with aquatic microbial communities. The study conclusively demonstrates that the antimicrobial efficacy of CuO nanoparticles is primarily governed by their size, surface characteristics, and environmental conditions. The observed multi-modal mechanism of action, combining physical membrane disruption, ROS generation, and ionic zinc release, suggests a robust antimicrobial system that microorganisms may find difficult to develop resistance against.

However, the significant alterations in microbial community structure raise important ecological considerations. The selective pressure exerted by CuO nanomaterials could potentially disrupt essential ecosystem services provided by aquatic microorganisms. The differential response observed among various microbial groups indicates the need for careful consideration in applications where maintaining ecological balance is crucial.

These findings have important implications for both environmental safety and technological applications. While the strong antimicrobial properties of CuO nanomaterials show promise for water treatment applications, their deployment should be carefully managed to minimize ecological impact. Future research should focus on developing surface modifications that enhance selectivity towards pathogenic organisms while minimizing effects on beneficial microbes. Additionally, long-term studies on the evolution of microbial communities under CuO nanomaterial exposure are recommended to assess potential adaptation mechanisms and ecological stability.

This research contributes significantly to our understanding of nano-bio interactions in aquatic environments and provides a foundation for developing more sustainable nanomaterial-based water treatment technologies. The insights gained will be valuable for regulatory frameworks governing the use of nanomaterials in aquatic environments and will inform the design of next-generation antimicrobial materials.

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