



Toxicological And Environmental Perspectives Of Biogenic Silver Nanoparticles In Antibacterial Applications

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Abstract:

The growing prevalence of multidrug-resistant bacterial infections has necessitated the development of alternative antimicrobial strategies beyond conventional antibiotics. Nanotechnology has emerged as a powerful platform in this regard, with silver nanoparticles (AgNPs) demonstrating broad-spectrum antibacterial activity through mechanisms such as membrane disruption, biofilm inhibition, enzyme inactivation, and reactive oxygen species generation. Although chemically synthesized AgNPs are effective, their application is limited by issues including residual chemical toxicity, poor biocompatibility, environmental contamination, and high production costs. Biogenic or green-synthesized AgNPs, produced using plant extracts, bacteria, fungi, or algae, have gained significant attention as sustainable alternatives that align with green chemistry principles.

This review provides a comprehensive evaluation of biogenic silver nanoparticles from toxicological and environmental perspectives, with particular emphasis on their antibacterial applications. The synthesis mechanisms, physicochemical characteristics, and modes of antibacterial action of biogenic AgNPs are discussed alongside in vitro and in vivo toxicity profiles. Available evidence indicates that biomolecular capping agents derived from biological sources regulate silver ion release, thereby reducing cytotoxicity and enhancing biocompatibility compared to chemically synthesized counterparts. However, at higher concentrations, biogenic AgNPs may still induce oxidative stress, DNA damage, and organ accumulation, highlighting the importance of dose optimization. The environmental fate and ecotoxicological impacts of biogenic AgNPs are also examined, including their aggregation, transformation, biodegradation, and effects on aquatic and soil organisms.

Finally, the review identifies regulatory challenges, risk assessment limitations, and key research gaps, particularly related to long-term exposure and lifecycle impacts. Overall, biogenic AgNPs represent promising antibacterial nanomaterials with improved safety and environmental profiles, provided that standardized testing, safe-by-design approaches, and responsible regulatory frameworks are implemented to support their sustainable biomedical and environmental use.

Index Terms - Biogenic silver nanoparticles; Green synthesis; Antibacterial activity; Toxicological assessment; Environmental impact.

1. INTRODUCTION

Nanotechnology has transformed antibacterial therapy by introducing nanomaterials that disrupt bacterial biofilms, inhibit enzyme activity, and generate reactive oxygen species for potent antimicrobial effects. Silver nanoparticles (AgNPs) hold particular significance due to their broad-spectrum efficacy against multidrug-resistant pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa*, outperforming conventional antibiotics in clinical isolates. These properties stem from AgNPs' high surface-to-volume ratio, enabling controlled ion release that damages bacterial membranes and DNA.

Chemically synthesized AgNPs, reliant on reducing agents like sodium borohydride and stabilizers such as polyvinylpyrrolidone, introduce limitations including cytotoxicity from residual toxins, inconsistent particle size distribution, and high production costs. These methods often yield nanoparticles prone to aggregation and environmental release of hazardous precursors, exacerbating pollution in water bodies.

Biogenic or green-synthesized AgNPs have emerged as sustainable alternatives, utilizing plant extracts (e.g., neem, aloe vera), bacteria, or fungi as bioreductants and capping agents to produce stable, polydisperse nanoparticles under mild conditions. For example, *Azadirachta indica*-mediated AgNPs demonstrate superior stability and antibacterial potency against *Escherichia coli* with reduced energy input compared to chemical routes. This shift aligns with green chemistry principles, minimizing waste and enhancing biocompatibility.

Despite these advances, toxicological and environmental assessments remain critical. Biogenic AgNPs can induce oxidative stress, DNA damage, and bioaccumulation in mammalian cells and aquatic organisms, while silver ion leaching poses risks to microbial diversity and food chains. Acute exposure studies report liver and spleen accumulation in rodents, alongside genotoxicity in human cell lines at concentrations above 50 µg/mL. This review aims to synthesize evidence on the toxicological profiles (in vitro/in vivo) and environmental fate of biogenic AgNPs in antibacterial contexts. Scope includes synthesis optimization, efficacy-toxicity correlations, ecological risk modeling, and regulatory gaps, drawing from 2020-2025 studies to inform safe biomedical translation [1, 2, 3, 4].

2.1. Concept of Biogenic (Green) Synthesis

Biogenic synthesis represents an eco-friendly paradigm in nanotechnology, leveraging biological entities such as plants, microbes, and algae to produce silver nanoparticles (AgNPs) without toxic chemicals. This green approach utilizes natural reducing agents like polyphenols, flavonoids, and enzymes, alongside capping biomolecules that ensure nanoparticle stability and monodispersity under ambient conditions.

Plant-mediated synthesis dominates due to its simplicity and scalability; leaf extracts from species like *Azadirachta indica* or *Camellia sinensis* reduce silver ions (Ag⁺) to zerovalent AgNPs (10-50 nm), yielding spherical particles with enhanced bioavailability. Microbial routes employ bacteria (*Bacillus subtilis*) or fungi (*Aspergillus niger*), where extracellular enzymes facilitate bioreduction, producing polydisperse AgNPs with inherent antimicrobial coatings.

Compared to chemical methods using sodium borohydride, biogenic synthesis eliminates hazardous byproducts, operates at neutral pH and room temperature, and aligns with 12 principles of green chemistry by minimizing energy and waste. The biomolecules impart biocompatibility, reducing cytotoxicity while preserving potent antibacterial action via membrane disruption and ROS generation.

Key advantages include cost-effectiveness, renewability, and reduced environmental footprint, though challenges persist in standardizing extract composition across batches. This method underpins sustainable AgNP production for antibacterial textiles, wound dressings, and water purification [1, 2, 5, 6].

2.3 Biogenic silver nanoparticles (AgNPs) offer sustainable alternatives to chemical synthesis for antibacterial applications, leveraging biological sources to minimize environmental toxicity. Plant-based synthesis utilizes phytochemicals like flavonoids and terpenoids from extracts of leaves, roots, or fruits (e.g., Aloe vera, *Azadirachta indica*) as reducing and capping agents, yielding stable AgNPs with sizes of 10-50 nm that exhibit potent activity against multidrug-resistant pathogens via membrane disruption and reactive oxygen species generation. Microbial synthesis employs bacteria (e.g., *Bacillus subtilis*), fungi (e.g., *Aspergillus niger*), and algae (e.g., *Sargassum* sp.), where enzymes such as nitrate reductase facilitate extracellular or intracellular Ag⁺ reduction, producing polydisperse nanoparticles with enhanced biocompatibility and lower cytotoxicity compared to synthetic counterparts. Enzyme-mediated synthesis, often using isolated oxidoreductases or cell-free extracts, provides precise control over particle morphology, enabling tailored antibacterial efficacy while mitigating ecological risks like bioaccumulation in aquatic systems. These green methods address toxicological concerns by avoiding hazardous reagents, though challenges persist in scalability and long-term

stability. Overall, biogenic AgNPs balance efficacy and safety for environmental remediation and biomedical use.[7 8]

2.2 MECHANISM OF BIOGENIC AGNP FORMATION

Biogenic silver nanoparticles (AgNPs) form via a green, multi-stage process that underpins their low-toxicity profile for antibacterial applications. The primary step entails enzymatic or phytochemical reduction of Ag^+ ions from silver nitrate to Ag^0 atoms, mediated by NADH-dependent nitrate reductase in microbes or polyphenols in plant extracts, which donate electrons while avoiding harsh chemical reductants and thus limiting environmental release of toxic intermediates. Nucleation ensues as Ag^0 monomers aggregate into clusters, driven by thermodynamics and influenced by pH (optimal 7-9) and temperature (25-60°C); this rapid phase dictates initial particle size (often 5-20 nm), with controlled conditions yielding monodisperse nuclei for superior membrane-disrupting antibacterial action. Growth proceeds through Ostwald ripening or coalescence, expanding clusters into stable nanostructures like spheres or rods, where reaction kinetics fine-tune morphology to enhance pathogen targeting while minimizing aggregation-related sedimentation in ecosystems. Finally, capping and stabilization occur via adsorption of biomolecules—proteins, flavonoids, or polysaccharides—forming a corona that imparts steric/electrostatic barriers, boosting colloidal longevity, biocompatibility, and reduced bioaccumulation risks in aquatic biota. These mechanisms ensure biogenic AgNPs' eco-safety over synthetic analogs.[7.8]

2.3 Physicochemical Characteristics of Biogenic AgNPs

Biogenic silver nanoparticles (AgNPs) exhibit distinct physicochemical traits that underpin their efficacy and safety in antibacterial roles. Particle size typically spans 5-100 nm, with shapes ranging from spherical to anisotropic (rods, triangles), dictated by biological reductants; smaller sizes (<50 nm) enhance surface area for superior membrane permeation in bacteria, while negative surface charge (zeta potential -20 to -40 mV) from biomolecule adsorption boosts colloidal stability and reduces nonspecific protein binding. Crystallinity, often face-centered cubic as confirmed by XRD, alongside surface chemistry rich in hydroxyl, carbonyl, and amine groups (via FTIR), enables targeted interactions—crystalline facets like {111} amplify reactive oxygen species generation against pathogens without excessive dissolution in ecosystems. These attributes influence biological interactions profoundly: negatively charged, protein-capped surfaces minimize cytotoxicity to mammalian cells by evading immune clearance, yet promote bacterial adhesion and lipid peroxidation; shape-dependent uptake (e.g., rods > spheres) heightens selectivity for Gram-negative strains, mitigating environmental risks like algal toxicity through controlled ion release. Tailoring these properties via synthesis parameters addresses toxicological concerns, favoring sustainable antibacterial deployment over chemically derived AgNPs.[7.8.9.10].

3. Antibacterial Applications of Biogenic Silver Nanoparticles

3.1 Mechanisms of Antibacterial Action

Biogenic silver nanoparticles (AgNPs) combat bacterial pathogens through multifaceted mechanisms, offering superior efficacy against multidrug-resistant strains with reduced environmental toxicity. Cell membrane disruption occurs as negatively charged AgNPs adhere electrostatically to lipopolysaccharide layers in Gram-negative bacteria or peptidoglycan in Gram-positive ones, causing pit formation, lipid peroxidation, and cytoplasmic leakage, which halts cellular respiration. Concurrently, reactive oxygen species (ROS) generation—superoxide anions and hydroxyl radicals stems from electron transfer on AgNP surfaces, oxidizing membrane proteins and inducing oxidative stress that overwhelms bacterial antioxidant defenses like superoxide dismutase. DNA and protein damage follows: AgNPs penetrate cells, releasing Ag^+ ions that bind thiol groups in enzymes, inhibiting respiratory chains (e.g., NADH dehydrogenase), while intercalating into DNA to block replication and transcription, evidenced by comet assays showing genotoxicity in *E. coli* and *S. aureus*. Silver ion release, modulated by biomolecular corona dissolution, sustains intracellular toxicity yet minimizes ecological persistence compared to ionic silver, as capping agents limit uncontrolled leaching in soil and water systems. These synergistic actions ensure broad-spectrum activity, addressing toxicological concerns through targeted delivery.[7.8.9].

3.2 Activity Against Gram-Positive and Gram-Negative Bacteria

Biogenic silver nanoparticles (AgNPs) demonstrate robust comparative antibacterial efficacy against both Gram-positive and Gram-negative bacteria, with nuanced differences tied to cell wall architecture and nanoparticle properties. Gram-negative bacteria (e.g., *Escherichia coli*, *Pseudomonas aeruginosa*) often exhibit higher susceptibility due to thinner peptidoglycan layers and outer membrane lipopolysaccharides, facilitating AgNP adhesion, membrane disruption, and Ag⁺ influx; minimum inhibitory concentrations (MICs) typically range 1-10 µg/mL, outperforming Gram-positive counterparts by 2-5 fold in zone inhibition assays. Conversely, Gram-positive bacteria (e.g., *Staphylococcus aureus*, *Bacillus subtilis*) possess thicker peptidoglycan walls that impede penetration, yielding higher MICs (5-50 µg/mL), yet biogenic AgNPs' biomolecular corona enhances uptake via opsonin-like interactions, achieving >90% viability reduction at 20 µg/mL. Plant- and microbe-derived AgNPs (10-50 nm, ζ-potential -20 to -40 mV) excel against multidrug-resistant strains, with efficacy modulated by size (smaller particles superior) and shape (spheres > rods for Gram-negatives); synergistic effects with antibiotics amplify potency 4-10x, minimizing resistance development. Fungal-synthesized AgNPs show balanced activity (MIC ~2 µg/mL for both types), attributed to stable capping reducing ion burst release. Algal AgNPs favor Gram-positives via ROS overload, with 15 mm inhibition zones vs. *S. aureus*. These profiles underscore lower ecotoxicity, as controlled release limits soil/water persistence.[7.8.9.10.11]

3.3 Studies on In Vivo Toxicity

In vivo, biogenic silver nanoparticles (AgNPs) show dose-

dependent toxicity profiles that weigh the advantages of antibacterial therapy against possible hazards. Plant-capped AgNPs exhibit larger LD50 (>2000 mg/kg) than chemically produced counterparts because biomolecular stability limits rapid Ag⁺ release. However, acute toxicity appears at high doses (>50 mg/kg), producing temporary liver enzyme increase (ALT/AST >2x control) and hemolysis in mice. In rats, prolonged exposure (28-90 days, 1-10 mg/kg) causes mild splenic hyperplasia and renal tubular dilatation without overt mortality; in contrast to ionic silver, algal-derived AgNPs exhibit lower bioaccumulation (liver burden <5 µg/g), which is explained by fecal excretion and corona-mediated clearance. Through RES uptake, organ distribution favors the liver (40-60%), spleen (10-20%), and kidneys (5-15%); brain penetration is negligible (<1%) unless it is smaller than 20 nm; fungal AgNPs accumulate [12.13.14.15]

3.4 Pharmaceutical and Biomedical Uses

Because of their strong antibacterial activity, regulated ion release, and biocompatibility, biogenic silver nanoparticles (AgNPs) outperform their synthetic counterparts in biomedical applications. By eliminating *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms, lowering inflammation through ROS modulation, and encouraging collagen deposition, plant-derived AgNPs (such as those derived from Aloe vera) embedded in hydrogels speed up wound healing; clinical trials report 40-60% faster epithelialization with less than 5% cytotoxicity. Fungal-synthesized AgNPs are used in coatings for medical devices, such as implants and catheters, which produce >99% bacterial reduction over 30 days without leaching more than 1 µg/cm², preventing device-related infections while maintaining endothelial cell viability. With MICs of 2-8 µg/mL against resistant bacteria, topical preparations, such as creams containing algal AgNPs, heal atopic dermatitis and chronic ulcers; their biomolecular [7.8.9.11]

4. Biogenic Silver Nanoparticles from a Toxicological Perspective

4.1 The Value of Toxicological Assessment

Given their nanoscale interactions with biological systems, toxicological examination of biogenic silver nanoparticles (AgNPs) is crucial to ensuring safe translation from antibacterial efficacy to therapeutic and environmental applications. Extensive evaluations, including cytotoxicity (MTT, LDH assays), genotoxicity (comet assay), oxidative stress (ROS/DCF-DA), and in vivo histopathology, show that biomolecular capping

lessens acute toxicity when compared to chemical AgNPs; however, dose-dependent risks, such as DNA strand breaks and mitochondrial dysfunction, continue at $>50 \mu\text{g/mL}$ in mammalian cells. These analyses pinpoint crucial factors, such as particle size (less than 50 nm increases absorption), zeta potential (negative charges restrict hemolysis), and corona makeup (plant metabolites provide biocompatibility), which direct safer design in the context of growing biomedical use. Without thorough testing in accordance with OECD norms (such as 407/408 for subacute/chronic), inadvertent [15.16.17.18]

4.2 Studies on In Vitro Toxicity

Biogenic silver nanoparticles (AgNPs) exhibit complex dose- and cell-type-dependent effects in in vitro toxicity studies, which are essential for the safe application of antibacterial agents. Cytotoxicity assays (MTT, Alamar Blue) on mammalian lines like HEK-293, A549, and RAW 264.7 demonstrate IC_{50} values of 20-100 $\mu\text{g/mL}$ after 24-48 h, higher than chemical AgNPs (5-50 $\mu\text{g/mL}$), owing to protein/polyphenol capping that curbs Ag^+ burst release and lysosomal overload. Smaller particles ($<30 \text{ nm}$) induce greater membrane damage via lipid peroxidation, yet plant-derived AgNPs preserve $>80\%$ viability at therapeutic doses (10 $\mu\text{g/mL}$) by modulating corona interactions. Oxidative stress markers—elevated ROS (DCFH-DA), depleted GSH, and upregulated Nrf2—trigger mitochondrial depolarization and caspase-3 activation, driving apoptosis in HepG2 cells at 50 $\mu\text{g/mL}$, though fungal AgNPs attenuate this via inherent antioxidant flavonoids. Genotoxicity [7.8.17]

4. Biogenic Silver Nanoparticles from a Toxicological Perspective

4.3 Studies on In Vivo Toxicity

Biogenic silver nanoparticles (AgNPs) have acceptable safety profiles at therapeutic levels, which is important for antibacterial applications, according to in vivo toxicity studies. In contrast to chemical AgNPs, which cause rapid systemic toxicity due to uncontrolled ion release, plant-mediated AgNPs only cause transient hepatic enzyme elevations (ALT/AST $<3\times$ baseline) and no mortality at 2000 mg/kg. Acute toxicity testing in rodents (OECD 423) reveals high LD_{50} values ($>1000 \text{ mg/kg}$ oral/i.p.). Fungal-derived AgNPs demonstrate quicker clearance (half-life <7 days) via hepatobiliary channels, decreasing residual loads; chronic studies (90-day OECD 408) at 1-10 mg/kg reveal modest nephrotoxicity (tubular casts in 10-20% of rats) and splenic sequestration without carcinogenicity. Reticuloendothelial accumulation is more common in the liver (45–65%), spleen (15–25%), and lungs (5–10%), as measured by ICP-MS, with less than 2% [7.8.9.13.]

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greater in the liver and kidneys (ICPMS: 2030 $\mu\text{g/g}$ at 10 mg/kg/4.4 Comparative Toxicity of Chemically Synthesized and Biogenic AgNPs

Because of variations in surface chemistry and ion release kinetics, biogenic silver nanoparticles (AgNPs) exhibit significantly lower toxicity than their chemically manufactured counterparts. In HEK293 cells, chemical AgNPs stabilized by citrate or PVP show IC_{50} values of 5–

20 $\mu\text{g/mL}$ due to rapid Ag^+ dissolution ($>50\%$ in 24 h), which causes lysosomal rupture, ROS overload, and 40–

60% apoptosis; in contrast, biogenic AgNPs show $\text{IC}_{50} >50 \mu\text{g/mL}$ with $<20\%$ ion release because of polyphenol/protein coronas that buffer interactions and prolong half-life.

In vivo, chemical versions accumulate (35g), causing glomerular damage and ALT rise ($>5\times$), whereas plant-mediated AgNPs clear 70% faster through stool, reducing oxidative histopathology. From a Toxicological Point of View, Biogenic Silver Nanoparticles.

4.4 Comparative Toxicity of Biogenic and Chemically Synthesized AgNPs

Biogenic silver nanoparticles (AgNPs) are much less hazardous than their chemically produced counterparts due to differences in surface chemistry and ion release kinetics. In HEK-293 cells, chemical AgNPs stabilized by citrate or PVP show IC_{50} values of 5–20 $\mu\text{g/mL}$ due to rapid Ag^+ dissolution ($>50\%$ in 24 h), which causes lysosomal rupture, ROS overload, and 40–60% apoptosis; in contrast, biogenic AgNPs show $\text{IC}_{50} >50 \mu\text{g/mL}$ with $<20\%$ ion release because of polyphenol/protein coronas that buffer interactions and prolong half-life.

In vivo, chemical versions accumulate 3–5 times greater in the liver and kidneys (ICP-MS: 20–30 µg/g at 10 mg/kg), causing glomerular damage and ALT rise (>5x), whereas plant-mediated AgNPs clear 70% faster through stool, reducing oxidative histopathology. [7,8,9,13]

4.5 Factors Influencing Toxicity

Toxicity of biogenic silver nanoparticles (AgNPs) hinges on intrinsic physicochemical properties and exposure parameters, dictating risk in antibacterial contexts. Particle size profoundly impacts biodistribution: nanoparticles <20 nm exhibit 3-5x higher cellular uptake via clathrin-mediated endocytosis, elevating ROS and mitochondrial dysfunction in hepatocytes (IC₅₀ ~10 µg/mL), whereas >50 nm variants show reduced penetration and lower cytotoxicity due to sedimentation. Shape modulates interactions—spherical AgNPs induce milder membrane perturbation than sharp-edged rods or triangles, which amplify physical damage and LDH release by 40% in A549 cells. Dose-response follows a non-linear threshold: acute exposures >50 µg/mL trigger apoptosis via caspase-3, while chronic low-doses (1-5 µg/mL, >14 days) provoke adaptive Nrf2 upregulation, mitigating oxidative stress; prolonged exposure duration correlates with cumulative Ag⁺ burdens and genotoxicity (comet tail moment +25%). Surface functionalization via biogenic capping—flavonoids, proteins—sterically hinders protein corona formation, slashing inflammatory cytokines (IL-6 -50%) and hemolytic potential compared to citrate-stabilized AgNPs. Route of exposure alters kinetics: dermal applications limit systemic access (<1% bioavailability), unlike intravenous routes yielding peak liver accumulation (30 µg/g at 10 mg/kg), underscoring application-specific risk mitigation. Optimizing these factors enhances safety margins for environmental deployment. [7,8,9,13,17]

Environmental Perspectives of Biogenic Silver Nanoparticles

5.1 Environmental Release and Exposure Pathways

Biogenic silver nanoparticles (AgNPs) enter ecosystems primarily through medical/industrial waste and agricultural/water systems, necessitating toxicity risk assessments for sustainable antibacterial use. Medical waste from wound dressings and device coatings—containing 0.1-5% biogenic AgNPs releases via incineration leachates or landfill runoff, with studies detecting 10-200 ng/L in hospital effluents; plant-capped AgNPs transform to less bioavailable Ag₂S (sulfidation >70% in sludge), curbing aquatic dispersion compared to synthetic forms. Industrial effluents from textile/pharmaceutical production discharge 1-50 µg/L AgNPs, aggregating in wastewater treatment plants (removal efficiency 90-99%) yet persisting as nanoparticulate residues in biosolids applied to farmland. Agricultural pathways involve biosolid-amended soils (10-100 mg Ag/kg dry weight), where AgNPs uptake by crops like wheat (0.1-2 µg/g in shoots) occurs via root endocytosis, potentially entering food chains; microbial capping accelerates soil retention, minimizing groundwater leaching (<5% mobility). In water systems, disinfection filters and runoff introduce 5-20 µg/L, inactivating coliforms yet posing risks to algae (*Chlorella sp.* EC₅₀ 15 µg/L) and daphnids (LC₅₀ 20 µg/L); however, biogenic coronas promote sedimentation and microbial degradation, reducing trophic transfer. These pathways underscore controlled-release designs to limit bioaccumulation. [7,8,9,10,19]

5.2 Fate and Transport in the Environment

Biogenic silver nanoparticles (AgNPs) undergo dynamic fate and transport processes in environmental compartments, influenced by their biomolecular corona that modulates reactivity. Aggregation occurs rapidly in high ionic strength media (e.g., seawater, Ca²⁺/Mg²⁺ >10 mM), forming micrometer-sized clusters within hours (attachment efficiency α >0.1), accelerated by neutral pH but retarded by protein/ polysaccharide capping that imparts steric stabilization; this reduces mobility yet limits bioavailability to pelagic organisms. Dissolution yields Ag⁺ at 1-5% initially, suppressed 3-10x compared to citrate-AgNPs by sulfide complexation and surface passivation, with equilibrium [Ag⁺] <1 µg/L under anoxic conditions; sulfidation to Ag₂S dominates in sediments (transformation half-life 2-7 days), yielding insoluble phases (K_{sp} 6×10⁻⁵⁰) that immobilize 80-95% of released silver. In soils, interaction with humic acids and clays promotes retention (K_d >10³ L/kg), with <5% leaching through macropores; microbial exudates enhance heteroaggregation, confining transport to <10 cm depth in agricultural fields. Aquatic components—DOM, phosphates form labile coronas that slow settling (sedimentation rate 0.1-1 m/day) while attenuating toxicity to algae via ion scavenging; biogenic AgNPs exhibit 2-4x lower EC₅₀ in *Daphnia magna* (25 µg/L) due to controlled dissolution. These processes favor localized persistence over long-range dispersion [7,8,9]

5.3 Ecotoxicological Effects

Biogenic silver nanoparticles (AgNPs) exhibit moderated ecotoxicity compared to synthetic variants, attributed to biomolecular capping that limits Ag^+ bioavailability across trophic levels. In aquatic organisms, plant-derived AgNPs induce LC50 values of 20-50 $\mu\text{g/L}$ in *Daphnia magna* (48 h) and *Thamnocephalus platyurus*, 2-4x higher than chemical AgNPs due to suppressed dissolution (<5% vs. 30%); algal species (*Chlorella vulgaris*) show EC50 >100 $\mu\text{g/L}$ with inhibited photosynthesis (PSII yield -25%) from ROS, yet recover post-exposure via corona-mediated sedimentation. Fish (*Oryzias latipes*) display gill hyperplasia at 10 $\mu\text{g/L}$ chronic exposure, but fungal AgNPs reduce bioavailability through protein complexation, limiting Na^+/K^+ -ATPase disruption and bioaccumulation factors (<1 in muscle). Soil microorganisms face dose-dependent impacts: nitrogen fixation by *Azotobacter* declines 30-50% at 10 mg/kg, while denitrification rates drop via nitrate reductase inhibition; however, biogenic capping preserves 70-80% microbial diversity in long-term assays by curbing ion efflux, unlike citrate-AgNPs that halve respiration (CLPP -60%). Phytotoxicity manifests as root elongation inhibition (20-40% at 50 mg/kg soil) in *Lactuca sativa* and wheat, linked to Ag^+ uptake and mitotic arrest, though polysaccharide coats mitigate oxidative bursts in shoots (MDA levels <2 nmol/g), promoting 15% better germination than uncapped forms. These effects underscore context-specific risk mitigation.[7,8]

5.4 Biodegradability and Environmental Stability

Biogenic silver nanoparticles (AgNPs) demonstrate enhanced biodegradability and controlled environmental stability compared to synthetic counterparts, driven by their biological coatings that facilitate natural degradation pathways. Persistence varies by matrix: in soils, plant-derived AgNPs exhibit half-lives of 10-30 days through microbial sulfidation to insoluble Ag_2S (conversion >80%), substantially shorter than chemical AgNPs (>90 days) due to enzymatic reduction by soil bacteria like *Pseudomonas sp.*. In aquatic systems, aggregation and sedimentation dominate within 48 hours (rate 0.5-2 m/day), with dissolution limited to <3% Ag^+ release under oxic conditions, as biomolecules chelate ions and promote biofilm-mediated biotransformation. Biological coatings—flavonoids, proteins, polysaccharides—play crucial roles in degradation: polyphenols act as electron donors for dissimilatory metal reduction, accelerating $\text{Ag}^0 \rightarrow \text{Ag}_2\text{S}$ by 4-6x; fungal mannans foster heteroaggregation with natural organic matter, enhancing gravitational settling; algal sulfated polysaccharides catalyze photoreduction under UV, yielding elemental silver occlusion. These coatings confer "soft" dissolution kinetics, maintaining ecotoxicity below chronic NOEC (10 $\mu\text{g/L}$ for *Daphnia*) while enabling complete mineralization within 60 days in activated sludge (COD removal >95%). Such engineered lability balances antibacterial utility against long-term accumulation risks. [7,8]

6. Risk Assessment and Regulatory Considerations

Biogenic silver nanoparticles (AgNPs) present unique toxicological challenges due to their size-dependent reactivity and environmental persistence in antibacterial applications. Existing guidelines for nanomaterials, such as those from the European Chemicals Agency (ECHA) and the U.S. Environmental Protection Agency (EPA), classify AgNPs as novel entities requiring specific registration under REACH and TSCA frameworks, emphasizing exposure limits and labeling. These regulations address acute cytotoxicity and genotoxicity observed in vitro, yet fall short for biogenic variants coated with biomolecules that alter bioavailability. |

Challenges in AgNP regulation stem from variability in synthesis methods, particle morphology, and release profiles, complicating dose-response modeling across ecosystems. Physicochemical heterogeneity hinders uniform safety thresholds, as smaller biogenic AgNPs (<50 nm) exhibit higher ion dissolution and bioaccumulation in aquatic organisms.

Standardized toxicity protocols are urgently needed, integrating OECD test guidelines with nanoparticle-specific endpoints like agglomeration and corona formation. Current assays often overlook chronic exposure and trophic transfer, necessitating harmonized in vivo models. |

Environmental risk assessment models, such as the SimpleBox4nano and probabilistic risk assessment tools, predict AgNP fate in soil and water compartments. These models highlight sediment accumulation as a

primary concern, urging lifecycle analyses for antibacterial products to mitigate ecological impacts. [20,21,22,23,24]

7. Strategies to Minimize Toxicity and Environmental Impact

Optimization of biogenic synthesis reduces toxicity by selecting microbial or plant extracts that yield stable, monodisperse silver nanoparticles (AgNPs) with natural capping agents, limiting uncontrolled ion release. Parameters like pH, temperature, and precursor concentration can be tuned to produce larger particles (>50 nm) with lower reactivity, enhancing biocompatibility for antibacterial use.

Surface modification through functionalization with polysaccharides or peptides from biogenic sources stabilizes AgNPs, preventing agglomeration and reducing cellular uptake in non-target organisms. Controlled release mechanisms, such as embedding in biopolymer matrices like chitosan, enable sustained antibacterial action while minimizing acute environmental exposure.

Dose minimization approaches employ lowest effective concentrations via combinatorial therapies with antibiotics, achieving synergistic effects that lower AgNP requirements by up to 80%. This strategy curbs bioaccumulation in aquatic systems.

Safe-by-design nanomaterials integrate toxicity-reducing features from inception, including degradable coatings and targeted delivery for wound dressings, aligning with green nanotechnology principles. Lifecycle assessments guide these designs to ensure end-of-life breakdown without persistent residues. [25,26,27,28]

8. Current Challenges and Research Gaps

Biogenic silver nanoparticles (AgNPs) face significant hurdles in toxicological evaluation due to the lack of long-term toxicity data spanning multi-generational exposure in mammals and ecosystems. Most studies focus on acute effects like oxidative stress and cytotoxicity within 72 hours, leaving chronic bioaccumulation, epigenetic alterations, and reproductive impacts underexplored in realistic environmental scenarios.

Inconsistencies in experimental models exacerbate this gap, with variations in particle size, capping agent composition, and exposure media yielding conflicting results across cell lines, zebrafish embryos, and soil invertebrates. Biogenic coatings from diverse plant extracts introduce batch-to-batch heterogeneity, undermining comparability and meta-analyses.

Scale-up and reproducibility issues hinder industrial translation, as laboratory bioreactors fail to replicate microbial or plant-mediated synthesis at tonnage levels without compromising monodispersity or bioactivity. Standardization of precursors and process controls remains elusive.

Limited environmental lifecycle studies overlook transformation pathways sulfidation, chlorination, and homoaggregation in wastewater treatment plants and sediments, impeding predictive modeling of persistence and trophic transfer. Comprehensive cradle-to-grave assessments integrating probabilistic exposure are urgently required. [32,33]

9. Future Perspectives

Sustainable nanotechnology development prioritizes biogenic silver nanoparticles (AgNPs) synthesized via plant or microbial routes to minimize chemical inputs and enhance biodegradability in antibacterial applications. Advances in reactor design and extract standardization promise scalable production with reduced ecological footprints, aligning with circular economy principles.

Integration with antimicrobial stewardship programs optimizes AgNP deployment in healthcare settings, combining them with conventional antibiotics to combat resistance while limiting selective pressure. This approach supports precision medicine, where AgNP coatings on medical devices provide on-demand release triggered by infection biomarkers.

Translation from lab to clinical and environmental safety requires bridging efficacy demonstrations with rigorous toxicological profiling. Phase III trials and real-world monitoring will validate long-term safety, incorporating digital twins for predictive modeling of AgNP fate in vivo and ecosystems. Regulatory sandboxes facilitate accelerated approval for "safe-and-effective" biogenic formulations, addressing gaps in current nanomedicine guidelines.

Multidisciplinary efforts, including AI-driven synthesis optimization and global harmonization of risk assessment, will drive clinical adoption. These strategies ensure biogenic AgNPs evolve as environmentally benign alternatives in the global fight against antimicrobial resistance.[34.35.36.37]

10. Conclusion

Biogenic silver nanoparticles (AgNPs) demonstrate superior antibacterial efficacy against multidrug-resistant pathogens, disrupting biofilms and membrane integrity through multifaceted mechanisms including reactive oxygen species generation and ion release. Their eco-friendly synthesis using plant or microbial extracts yields stabilized formulations with enhanced biocompatibility compared to chemically derived counterparts, positioning them as promising alternatives in wound dressings, coatings, and therapeutics.

Despite these benefits, toxicity concerns persist, encompassing cellular oxidative damage, genotoxicity, and environmental bioaccumulation in aquatic and soil compartments. Biogenic capping partially mitigates risks by controlling dissolution rates, yet long-term ecological impacts and human exposure thresholds demand vigilant monitoring.

Responsible implementation mandates lifecycle assessments, standardized protocols, and safe-by-design principles to balance therapeutic gains against potential hazards. Sustainable production scales, regulatory harmonization, and stewardship integration will ensure biogenic AgNPs contribute meaningfully to antimicrobial innovation without compromising ecosystems or public health.[38,39,40,41]

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