

Tri-metallic Nanoparticles: A Green Approach to Multifaceted Biological Applications

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Abstract

Trimetallic nanoparticles (TNPs) with three metallic elements show improved properties in catalysis, optics, magnetism and biocompatibility as compared with the mono and bimetallic nanoparticles. The unique properties allow for application in agriculture (e.g., enhancing plant growth, controlling pathogens) and biomedicine (e.g., drug delivery, cancer treatment). Conventional methods of TNPs synthesis involve toxic reagents and consume a high amount of energy. However, green synthesis of TNPs using natural agents like plant extract is a sustainable approach. This green approach leads to the production of versatile nanoparticles with significant applications in improving agricultural methods and health outcomes.

Keywords: *Agricultural Application; Biological Application; Biomedical Application; Green Synthesis; Microorganism-Mediated Biosynthesis; Plant Mediated Biosynthesis; Trimetallic Nanoparticles*

Introduction

Nanotechnology has changed medicine, pharmaceuticals, materials and agriculture. TNPs have collected attention due to its unique physicochemical properties and synergistic effect, which often outperform mono- and bimetallic. TNPs are promising biomedical and agricultural candidates due to their improved catalytic activity, tunable optical and magnetic behavior, thermal stability and biocompatibility (Roy *et al.*, 2023).

Traditional TNPs synthesis uses hazardous reagents, high energy and harmful byproducts. A sustainable, cost-effective alternative is green synthesis, which uses plant extracts, bacteria, fungi and algae. They naturally reduce and cap TNPs, giving them biologically useful size, shape and surface properties (Radulescu *et al.*, 2023).

TNPs biologically synthesized by biologists are promising for targeted drug delivery, antimicrobials, cancer therapy, bioimaging, biosensing and tissue engineering. Green synthesis of three metals improves biological interactions, therapeutic efficiency and diagnostic performance while reducing toxicity (Kulkarni *et al.*, 2023; Vijayaram *et al.*, 2024). As scientists study TNPs' effects on plant health, soil quality and crop productivity, their use in agriculture is growing. Besides fighting phytopathogens, TNPs' catalytic and redox properties help crops deliver nutrients, degrade pesticides, germinate seeds and tolerate stress. For sustainable farming, biocompatibility and environmental responsiveness reduce agrochemical use and promote environmentally friendly agricultural systems (Osman *et al.*, 2024).

In biomedical and agricultural nanotechnology, biologically synthesized trimetallic nanoparticles offer novel solutions to healthcare, environmental sustainability and global food security.

Biological application of Trimetallic nanoparticles (TNPs)

The presence of three different metals in the same nanostructure enhances their physicochemical stability, catalytic activity and multifunctionality that are highly attractive for biomedical agricultural applications.

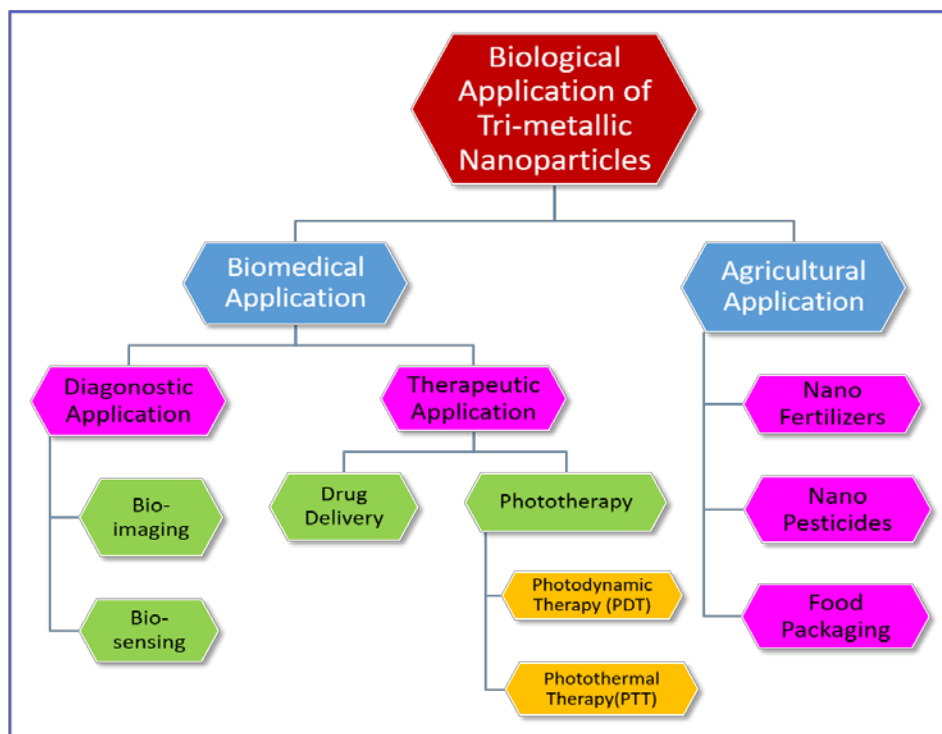


Figure 1: Biological Application of Trimetallic nanoparticles (TNPs)

In medicine, TNPs are studied for targeted drug delivery, antimicrobial therapy, bioimaging, biosensing and cancer therapy, where tunable surface chemistry and optical properties result in improved therapeutic efficacy and reduced toxicity.

In agriculture, TNPs are promising nanofertilizers, nanopesticides and antimicrobial agents for crop protection, thereby establishing the path to sustainable farming practices with a lesser chemical load. These advances together showcase the potential of TNPs as next-generation nanoplatforms for addressing global challenges in health and food security.

Biomedical Application

Anti-Cancer Activity:

Cancer remains a major killer worldwide; early diagnosis and treatment are very important. Biological and green synthesis methods for the synthesis of trimetallic nanoparticles demonstrate promising cytotoxic activity against a variety of cancer cell lines and are considered as promising novel anticancer agents.

Au/CuO/ZnO nanoparticles derived from *Verbena officinalis* (100% cytotoxicity in Jurkat cells at 6 μmol) (Dobrucka *et al.*, 2021) and Au/Pt/Ag nanoparticles derived from *Pleurotus florida* (10% viability decreases in MDA-MB-231 breast cancer cells at 100 $\mu\text{g/ml}$) demonstrated remarkable antileukemia (Chaturvedi *et al.*, 2020). While Ru/Ag/Pd nanoparticles synthesized from garlic tunicate leaves demonstrated antiproliferative effects against HepG2, Caco-2 and K562 cancer cell lines (Hussein *et al.*, 2022). Similarly, Ag/Co/Fe nanoparticles produced using *Citrus lemon* showed notable cytotoxicity against HepG2 and MCF7 cells (Abdelsattar *et al.*, 2023). Trimetallic nanostructures have also been employed for cancer diagnostics. Highly sensitive detection of H_2O_2 from cancer cells using Au-Pt-Pd/rGO nanocomposites and trimetallic hybrid nanoflower-decorated MoS_2 nanosheets (Dou *et al.*, 2018).

These results show the importance of trimetallic nanoparticles for cancer therapy and diagnostic applications.

Sensing Applications:

Biosensors are utilized for qualitative and quantitative detection of biomolecules and trimetallic nanoparticles (TNPs) have emerged as very useful sensing platforms because of their controllable plasmonic and magnetic properties. Plasmonic TNPs use localized surface plasmon resonance (LSPR), where shifts in wavelength occur due to change in refractive index or nanoparticle agglomeration, allowing sensitive analytic detection.

Trimetallic nanoparticles (TNPs) have demonstrated significant potential in biosensing and theranostic applications owing to their enhanced catalytic and optical properties. Cu-Au-Pt nanoparticles exhibited strong NIR-I plasmonic absorption and were successfully applied in glucose sensing, biosensing and photothermal cancer therapy. Au-Ag-Pd nanocomposites enabled the simultaneous detection of multiple biomolecules, including ascorbic acid, dopamine, acetaminophen and tryptophan (Abdelwahab *et al.*, 2020). Pd-Cu-Au nanoparticles showed excellent peroxidase-like activity, allowing ultrasensitive detection of H₂O₂ and glucose with detection limits of 5 nM and 25 nM, respectively (Nie *et al.*, 2020).

Furthermore, Au-Pt-Pd/reduced graphene oxide nanocomposites were used for monitoring H₂O₂ release from live cancer cells. While Pd-Fe-Ni alloy nanoparticles served as effective electrochemical sensors for biotin detection (Gholivand *et al.*, 2015). These findings highlight the versatility of trimetallic nanoparticles as promising platforms for advanced biosensing, diagnostic and theranostic applications.

Antimicrobial Activity:

TNPs showed good antibacterial activity through different mechanisms. They quickly bind to the cell walls of bacteria, disrupting the membrane integrity and causing intracellular components to leak out. In addition, the production of reactive oxygen species (ROS) leads to the oxidation damage of important biomolecules, while direct contact with proteins and DNA also inhibits the growth of bacteria (Slavin *et al.*, 2017). For example, Au-Ag-Cu TNPs showed a wide range of efficacy against both Gram-positive (*Staphylococcus aureus*) and Gram-negative *E. coli* bacteria (Markowska-Szczupak *et al.*, 2025). Similarly, Pt-Ag-Cu TNPs were found to be highly efficient against multidrug-resistant bacteria mainly due to their increased ROS-mediated killing of bacteria (Rai *et al.*, 2012). The results demonstrate the potential of trimetallic nanoparticles as new candidates of antimicrobial agents against drug-resistant bacterial infections.

Antifungal Activity:

Just like their antibacterial potential, TNPs exhibited strong inhibitory effects against pathogenic fungi. They interact with fungal cell walls and change membrane permeability, and their ability to bind intracellular proteins and nucleic acids interferes with metabolic processes and replication. Also, ROS overproduction promotes the apoptosis of the fungal cells (Rai *et al.*, 2017). For instance, Cu-Ag-ZnO TNPs have demonstrated notable inhibition of *Candida albicans* (Ghosh *et al.*, 2020). The results suggest that TNPs have the potential to be used as antifungal agents in both clinical and agriculture settings.

Antioxidant Activity:

In addition to their antimicrobial properties, TNPs display strong antioxidant potential. They effectively scavenge free radicals such as DPPH• and ABTS•+, either through electron transfer or hydrogen atom donation. The multimetallic composition provides enhanced redox potential, mimicking the catalytic activity of antioxidant enzymes such as superoxide dismutase and catalase (Phan-Xuan *et al.*, 2024). For example, Au-Ag-Pd TNPs exhibit superior radical-scavenging ability in DPPH and ABTS assays, while Cu-Fe-Ag TNPs significantly reduce oxidative stress by neutralizing ROS in vitro. This dual ability to combat both pathogens and oxidative stress renders TNPs highly promising for therapeutic applications.

Agricultural Application

TNPs can deliver multiple micronutrients, disease resistance and shelf life while reducing chemical load. Below are three main application areas with recent studies.

Nano Fertilizers:

The use of TNPs in agriculture is increasing as multifunctional nano fertilizers provide micronutrients and enrich plant resistance to biotic and abiotic stress. Completely trimetallic nano formulations are rare, but recent studies show the potential of combining multiple metal oxides. The CMC-Cu-Zn-Fe MNPs showed the potential as a nutrient carrier and nano pesticide, enhancing the tolerance to drought stress and surprising the fungal pathogens in tomato plants (Bouqellah *et al.*, 2023). Zinc-doped calcium phosphate nanoparticles ('nano Zn') also demonstrated improved nutrient supply (Ca, P and Zn) and an antibacterial effect against *Pseudomonas syringae*, with increases in nutrient uptake of up to 65% versus traditional ZnSO_4 . Another study reported that biogenic Cu/Ni/Co oxide TNPs combined with vermicompost with rice straw and press mud significantly increased germination and growth parameters of *Abelmoschus esculentus*, with metal accumulation within plant tissues remaining below WHO safety limits. Moreover, even if not at the nanoscale, a fortified N-P-K fertilizer with a tri-micronutrient matrix (Zn, Fe, Mg) showed evident benefits on tomato growth, underlining the agronomic relevance of the concurrent micronutrient supply and presenting a benchmark for forthcoming TNPs-based nano formulations (Venugopal & Rao, 2021). Collectively these findings highlight the potential of TNPs to enhance nutrient use efficiency, improve plant health and reduce dependence on traditional agrochemicals.

Nano Pesticides / Disease Management:

TNPs, a new class of multifunctional plant disease management agents, can be used to reduce the use of pesticides. For instance, the CMC-Cu-Zn-Fe magnetic nanoparticles have been reported to kill the *Fusarium oxysporum* in tomatoes and relieve drought stress (Bouqellah *et al.*, 2023). The green synthesized CuO-Ag/ZnO nanocomposites with *Ziziphus spina-christi* leaf extract showed broad-spectrum antimicrobial activity against clinical pathogens and also inhibition of virulence factors and ergosterol biosynthesis (5.9 nm) (El-Sawaf *et al.*, 2024). Their trimetallic structure provides to take advantage of their surface functionalization for phytogens. In addition, bimetallic Cu/Zn hybrid nanoparticles have shown to be highly effective against bacterial spot disease of tomato caused by *Xanthomonas spp.* This includes copper-tolerant strains, which demonstrate the benefit of combining materials with different mechanisms of action as antimicrobials. CMC-Cu-Zn-Fe NPs can also provide micronutrients (Cu, Zn, Fe) and thus exhibit dual functionality as nano pesticides and nano fertilizers, leading to reduced requirements of multiple agricultural inputs and enhanced plant health and productivity.

Food Packaging:

Antimicrobial food packaging with trimetallic or Mult metallic nanocomposite film can be used to reduce microbial contamination and extend the shelf life. Embedded films with trimetallic nanoparticles are rare, but some studies showed the advantages of blending metal or metal oxide nanoparticles in polymer matrices. The in-situ synthesized trimetallic Bi-Fe-Sn nanoparticles from *Terminalia arjuna* leaf extract showed significant antimicrobial activity against *Escherichia coli* and *Pseudomonas aeruginosa* in microcrystalline cellulose films, demonstrating that TNPs can be added to biodegradable packaging materials for surface protection (Dar *et al.*, 2023). The composite films with spinel nanoparticles ZnAl_2O_4 and CuAl_2O_4 and chitosan or shellac polymers showed enhanced mechanical strength, lower air permeability, and antibacterial properties against *Bacillus mycoides* and *Canida albicans* (Mohamed *et al.*, 2024).

While they are mixed metal oxides, rather than trimetallic nanoparticles, the structural and functional properties of these spinels make them useful analogs for multifunctional packaging films. A new method to

package grapes was based on chitosan films loaded with ZnO and Ag nanoparticles and citronella essential oil with broad-spectrum antimicrobial activity (Motelica *et al.*, 2020). The multi-component system demonstrates the synergistic formation of trimetallic or hybrid antimicrobial films by metallic nanoparticles and natural biocides. Active packaging films with silver in ZnO nanoparticles and catechin in chitosan/polyvinyl alcohol matrices protected strawberries for 12 days at 4°C, almost completely inhibiting *Staphylococcus aureus* and *E. coli* and improving barrier properties (Meshkini & Khoshshokhan, 2025). Non-trimetallic biohybrids demonstrate the potential of doping and multiple antimicrobial agents in food packaging.

Conclusion

Biosynthesis of trimetallic nanoparticles is an attractive green and sustainable approach for chemical and physical synthesis. Biologically synthesized trimetallic nanoparticles are more stable, synergistic and multifunctional than their monometallic and bimetallic counterparts. The biological agents decreased the environmental impact and enhanced the biocompatibility of these nanoparticles, which are therefore useful in catalysis, environmental remediation and biomedical applications. Green synthesis is a simple, efficient, and scalable approach for producing nanostructures. Further research is required to optimize the synthesis process and to determine their full practical applications.

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