

Sustainable Algae-Mediated Production of the Copper Oxide Nanoparticles: Progress, Prospects, and Environmental Applications

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Copper oxide nanoparticles (CuO NPs), renowned for their antimicrobial, antiviral, and antioxidant properties; have attracted substantial attention across diverse scientific and industrial domains. Their high surface-area-to-volume ratio, nanoscale size, and unique physicochemical characteristics position them as cost-effective alternatives to noble metals in applications such as drug delivery, environmental remediation, catalysis, wound healing, antimicrobial coatings, and biosensors. However, conventional CuO NP synthesis often involves toxic reducing agents, energy-intensive processes, and hazardous by-products—raising critical concerns regarding sustainability and environmental safety. To address these challenges, recent research has shifted toward green synthesis approaches using biological feedstocks. Among the most promising eco-friendly strategies are plant-mediated and algae-mediated synthesis techniques, which utilize naturally derived reducing and stabilizing agents to fabricate CuO NPs under mild, non-toxic conditions. Various algal species—including *Chlorella vulgaris*, *Sargassum muticum*, *Ulvalactuca*, and *Spirulina platensis*—offer abundant intracellular and extracellular metabolites (e.g., polysaccharides, proteins, phenolic) that drive bio-reduction and nanoparticle stabilization. Algae are regarded as more superior as compare to plant extract in the bio-fabrication of the nanoparticles due to their rich and consistent composition of the bioactive molecules that efficiently function as reducing and capping agents, leading to the faster nanoparticles formation with improved control over size, morphology and dispersion. Recent advancements include the implementation of continuous flow bioreactor systems, hybrid green-chemical methods, and scalable synthesis protocols that enhance nanoparticle yield, size control, and functional performance. Despite these promising developments, key challenges remain—such as standardizing biosynthetic conditions, ensuring reproducibility across scales, and fine-tuning particle morphology.

Keywords: Nanomaterials, CuO NPs, algae, green synthesis, environmental sustainability

Received: March 2026; Accepted: April 2026

Nanomaterials, especially metal oxide nanoparticles, have attracted considerable interest due to their unique size-dependent physicochemical characteristics and wide range technological applicability. Among these, copper oxides (CuO) nanoparticles have attracted significant interest because of their low-cost semiconducting properties and wide range of applications in catalysis, energy devices, and environmental remediation. Conventional synthesis approaches for CuO NPs frequently involve energy intensive process and hazardous chemicals, leading to concerns about environmental sustainability and scalability [1]. Consequently, green synthesis approaches have emerged as promising alternatives, with algae-mediated fabrication receiving growing interest due to its minimal resource requirement, rapid biomass growth, and abundant bioactive metabolites that serve as reducing and stabilizing agents. This review focuses

specifically on the algae-mediated green synthesis of CuO NPs, emphasizing their synthesis mechanism, physicochemical characteristics and functional applications [2]. The literature examined in this review comprises peer reviewed article published predominantly between 2015 and 2025, sourced from scopus, web of science and Google Scholar database using key words including “algae mediated synthesis”, “green synthesis of CuO nanoparticles”, and biogenic fabrication of CuO nanoparticles.

The high surface area to volume ratio of nanoparticles enhances various properties of nanomaterials, including biological activity, thermal conductivity, and chemical stability. Owing to their nonlinear optical properties, they hold significant value in diverse sectors such as medicine, agriculture, cosmetics, and food. Owing to these benefits, various

strategies have been established for the creation of nanomaterials encompassing physical, chemical, and biological techniques [3]. However, obstacles in the large-scale nanomaterials production, especially the dependence on environmentally hazardous chemicals, have encouraged the advancement of green synthesis techniques.

Nanomaterials may occur naturally, be formed as a by-product of a combustion process, or deliberately engineered to serve specific functions. These materials exhibited distinct physical and chemical properties compared to their bulk counterpart. To put this in perspective, a nanometer is one billionth of a meter. This size range is significant because at this scale, materials can exhibit unique physical, chemical, and biological properties that differ significantly from their bulk counterparts. Nanomaterials are the building blocks of a revolution happening at the tiniest scale [4]. Nanomaterials show extensive variations in shape, size, composition, and other features, allowing multiple classification approaches. A widely used approach is to categorize them according to their origin.

Natural Nanomaterials: These are the nano-scale materials that originate naturally without any human intervention.

Incidental Nanomaterials: These are the nanoscale materials that arise unintentionally as a byproduct of natural processes rather than being deliberately engineered

Engineered Nanomaterials: Engineered nanomaterials are intentionally created and fabricated at the nanoscale to exhibit unique properties and function for targeted applications.

Bio-inspired Nanomaterials: These are the materials developed at the nanoscale by emulating the structures, functions, or processes of biological systems to attain enhanced performance and functionality.

While engineered and bio-inspired nanomaterials attract significant research interest, they represent a small fraction of the total nanomaterial mass compared to naturally occurring ones. Despite their unique potential, widespread industrial use of engineered and bio-inspired nanomaterials is limited by challenges in large-scale production and thorough safety assessments [5]. Nanotechnology is a multidisciplinary field that blends engineering, chemistry, physics, biology, and medicine to manipulate matter at the atomic level. Working at the nanoscale—where one nanometer is the size of a hydrogen atom—allows us to create nanoparticles, nanomaterials, and nanostructures through chemical, molecular, and supramolecular techniques [6]. A key focus of nanotechnology is understanding how reducing a material's size, especially below 10 nanometers, drastically changes its properties compared to the bulk material. This size reduction significantly alters optical, electrical, and

magnetic characteristics [7]. For instance, gold nanoparticles 20 nanometers in diameter absorb light differently than 100-nanometer gold nanoparticles, demonstrating how size influences their optical behaviour. Nanotechnology is poised to be a transformative technology in the 21st century, enabling the practical application of nanoscience. It involves precisely manipulating matter at the nanometer scale—observing, measuring, assembling, controlling, and manufacturing to create useful products [8-9].

Distinct Properties and Benefits of Nanomaterials

Enhanced Surface Area-to-Volume Ratio: The increased surface area-to-volume ratio in nanomaterials is a primary reason for their altered properties.

Reactivity: Nanomaterials are often more reactive than bulk materials.

Optical properties: Nanoscale particles can interact with light in unique ways.

Mechanical Strength: Nanomaterials can exhibit exceptional strength and hardness.

Electrical Properties: Conductivity can be greatly altered at the nanoscale.

Comparative Study of Natural and Engineered Nanomaterials

Natural Nanomaterials

Understanding the role of naturally occurring nanomaterials is crucial for assessing their environmental impact. Research continues to explore how these materials influence geological and biological processes.

Engineered Nanomaterials

The development of engineered nanomaterials has accelerated rapidly, with applications in diverse sectors. "Top-down" and "bottom-up" approaches are used to create nanomaterials with tailored properties. The focus is on developing sustainable and safe nanomaterials for various applications [10].

Metal oxide nanoparticles (MOx) are a diverse class of nanomaterials with applications spanning chemistry, physics, and materials science. Many metals readily react with oxygen to form metal oxides with various structures. At the nanoscale, MOx exhibits unique properties, making it valuable in fluorescence and optical sensors, catalysis, photovoltaics, biomedicine, gas sensing, and fuel cells. The size and shape of these nanostructures significantly impact their optical, mechanical, electrical, magnetic, and catalytic properties [11]. Tailoring nanostructures for desired functionalities has driven interest in morphology-controlled synthesis. For instance, iron oxide nanoparticles exhibit size-dependent magnetic

behavior, with 55 nm Fe₂O₃ showing ferromagnetism, whereas 12 nm particles are paramagnetic. However, traditional synthesis techniques such as co-precipitation, milling, and lithography often struggle to control morphology, affecting conductivity and other properties. Model-assisted synthesis addresses these challenges by designing nanostructures with specific chemical and physical attributes. Several studies have explored template-assisted synthesis to fabricate well-defined MO_x nanomaterials, including 1D structures, porous films, and transition metal oxides [12]. Various chemical procedures are utilized for the generation of template-mediated MO_x nanostructure, including sonication, hydrothermal, electrochemical, chemical vapor deposition, and airless deposition. The various techniques, such as sintering, melting, or etching, were utilized in the final step of the generation of nanostructure to remove the template and yield pure nanostructure. This model-driven strategy exhibited a promising pathway for generating advanced MO_x nanoparticles with customized properties [13]. Bio-nanotechnology is a rapidly growing field where biological microorganisms play a prominent role in the fabrication of nanomaterials, leading to enhancing the characteristics of both microorganisms and developed nanomaterials. Biological fabrication relies on microbial enzymes and plant phytochemicals for oxidation and reduction [14]. Although physical and chemical methods are widely employed for the development of nanomaterials, they often suffer certain limitations, such as toxicity, low productivity, and adverse environmental impact. As a result, bio-fabrication is gaining attention as a green alternative [15]. This eco-friendly approach has successfully produced various metal and metal oxide nanoparticles, including silver (Ag), gold (Au), selenium (Se), platinum (Pt), zinc oxide (ZnO), iron oxide (Fe₂O₃), and graphene oxide. Among them, copper nanoparticles (CuNPs) are of growing interest due to their essential biological role. In humans, copper acts as a cofactor in enzymes regulating neuropeptides, antioxidant defense, and immune function, while in plants, it supports metabolic and physiological processes crucial for growth [16]. Copper oxide nanoparticles (CuO NPs) exhibit diverse properties such as antimicrobial,

catalytic, optical, superconductive, and gas-sensing capabilities. With a bandgap of 1.7 eV, CuO functions as a p-type semiconductor and holds promise as a bioactive agent. CuO NPs can be synthesized through various physicochemical techniques, including sol-gel, sonochemical, electrochemical, microwave-assisted, and solid-state reactions. Additionally, they can be biologically produced using algae, fungi, plants, and other biosynthetic methods (Table-1). The high catalytic reactivity, large surface area, and microbial interaction potential of Cu NPs/CuO NPs make them valuable in agriculture, biomedicine, textiles, and environmental applications [17].

Overview of the Common Approaches to the Fabrication CuO NPs

The development of CuO nanoparticles (CuO NPs) has advanced significantly over the past decade due to their industrial and biomedical applications. The development method plays a vital role as it directly influences the morphology, size, and overall properties of the final nanoparticles. CuO NPs demonstrate unique electrical, optical, and magnetic behavior distinct from their bulk counterparts (Figure 1).

Bottom-Up Approach

In this procedure, nanomaterials are developed from atomic or molecular building blocks through self-assembly, leading to the generation of nanostructures. Various chemical and biological techniques are employed for this purpose. In this process, the materials are reduced down into individual atoms before being combined via physical forces to produce larger, stable structures comprising colloidal solution-based nanoparticles or quantum dots (QDs). Changes in temperature, pH, plant extract concentration, metal salt concentration, and incubation time may influence the size, shape, and stability of nanomaterials. The method's capacity to generate uniform and precisely controlled nanoparticles makes it an effective approach for creating nanomaterials intended for use as such in pharmaceuticals, biosensors, medicine, and catalysis. [18].

Table 1. Comparison of the characteristic properties of CuO with other common nanoparticles.

S. No.	Name of Nanoparticles	Band Gap	Size	Application	Toxicity
1.	TiO ₂	3.0-3.9 eV	10-30 nm	Water Purification, electronics, cosmetics, agriculture	Cytotoxicity
2.	Ag	1.8-3.0 eV	10-50 nm	Antimicrobial, anticancer and antioxidants,	ecological toxicity
3.	ZnO	3-3.4 eV	20-60 nm	Drug delivery, wound healing, environmental remediation	ecological toxicity
4.	CuO	1.2 -2.1 eV	11-36 nm	Catalysis, energy storage, environmental remediation	ecological toxicity

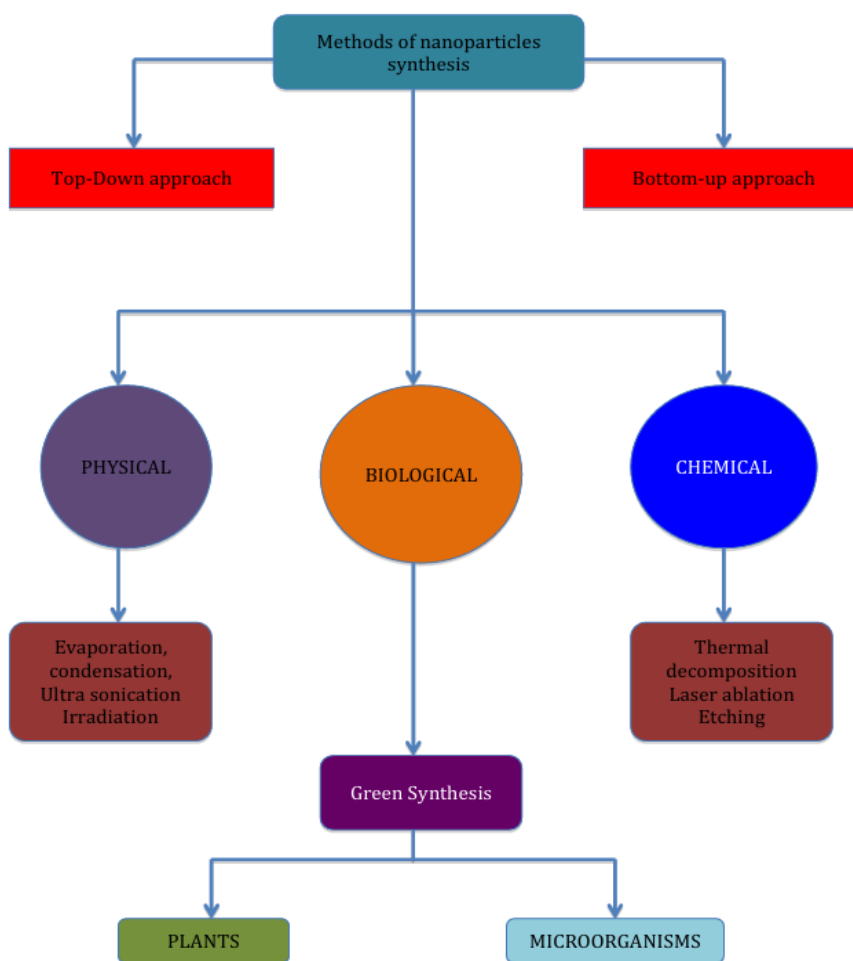


Figure 1. Various methods involve in the development of nanoparticles.

Top-Down Approach

Using a variety of physical methods, like lithography, crushing, and milling, bulk materials first undergo transformation into nanoparticles. By eliminating or manipulating thin layers of the bulk material, as in mask etching and ball milling, nanostructure creation can be externally controlled. Unlike the bottom-up approach, this method does not rely on molecular self-assembly but rather on the mechanical or physical reduction of larger materials. While effective, top-down methods may result in variations in the shape and size of the nanoparticles, requiring additional processing for uniformity. However, they are widely used in industries such as electronics and material science for fabricating nanoscale structures [19].

Green Synthesis of Nanoparticles

Green synthesis of CuO nanoparticles is ec-friendly low cost approaches that follow green chemistry principles, utilizing nontoxic solvent along with the

biological reducing and stabilizing agents to produce stable nanomaterials [20]. Unlike traditional physical and chemical methods which are often hazardous, energy intensive and environmentally harmful green synthesis employ plant, algae, bacteria and fungi to produce nanoparticles with controlled size and morphology while reducing environmental impact [21]. Among biological approaches plant mediated fabrication is widely preferred due to its simplicity, rapid reaction kinetics, and ability to generate high yield of stable nanoparticles [22]. Microbial synthesis involving bacteria and fungi offer advantages in terms of scalability and reproducibility but it typically requires longer cultivation time and more intricate handling [23, 24]. In contrast, algae mediated synthesis merge the strength of both approaches: it is rapid sustainable and cost effective with algae offering a diverse variety of bioactive metabolites that serve simultaneously as reducing and stabilizing agent. These characteristics features make algae as a highly promising platform for the green synthesis of CuO nanoparticles compared to the other biological system.

Algal Fabrication of CuO NPs

In the sustainability-focused era, researchers have widely pursued eco-friendly feedstock for a comprehensive and green biological approach for the creation of copper oxide nanomaterials. Broad spectrums of organisms, including plants, fungi, algae, and bacteria, naturally participate in the bio-fabrication of metal and metal oxide nanoparticles (Figure 2). This is accomplished by leveraging the bio-component of these organisms, including protein, lipids, and carbohydrates [25]. Among the various biological sources of the reducing agents for copper oxide nanoparticles, Algae are especially noteworthy for bio-fabrication due to their suitability as sustainable industrial resources. Algae are recognized for their rapid growth and can be cultivated using much less fertile land, fresh water, and pesticides compared to conventional plants. As a result, algae cultivation exerts minimal strain on critical human resources and has a negligible impact on the food chain. Moreover, algae are non-pathogenic organisms. Hence, Algae are regarded as an effective and reliable option for the green and sustainable production of copper oxide nanoparticles. For a long time, micro and macro algae have been employed in the human value chain, serving as a source of food, medicinal remedies, and fertilizers. Algae are typically classified according to their size and pigmentation. With respect to size, algae are categorized into macroalgae and microalgae.

Macroalgae, commonly referred to as seaweed, are multicellular organisms that can attain a substantial length, frequently exceeding 50 meters, and are visible without the aid of a microscope. Conversely, microalgae are tiny single-celled microorganisms that can exist alone or cluster together to form colonies. Moreover, microalgae vary in size, spanning from a few micrometers to several hundred micrometers depending on the species [26].

In the past two decades, researchers have emphasized the outstanding reducing ability of microalgae and macroalgae in the creation of metal oxide nanoparticles with remarkable stability. The field has advanced considerably through the exploration of diverse algae, which have been shown to facilitate the creation of nanoparticles by employing the biomolecules (Table 2). Algae have attracted attention in CuO NPs development owing to their abundance of bioactive compounds, which behave as natural reducing and stabilizing components. Brown algae species such as *Bifurcaria bifurcata* and *Cystoseira trinodis* have been used to produce CuO NPs ranging from 5 to 45 nm in size. Similarly, green algae like *Botryococcus braunii* and *Anabaena cylindrica* have been employed to generate nanoparticles under controlled conditions. However, further research is needed to identify the specific biomolecules involved in CuO NP formation and their potential applications [27].

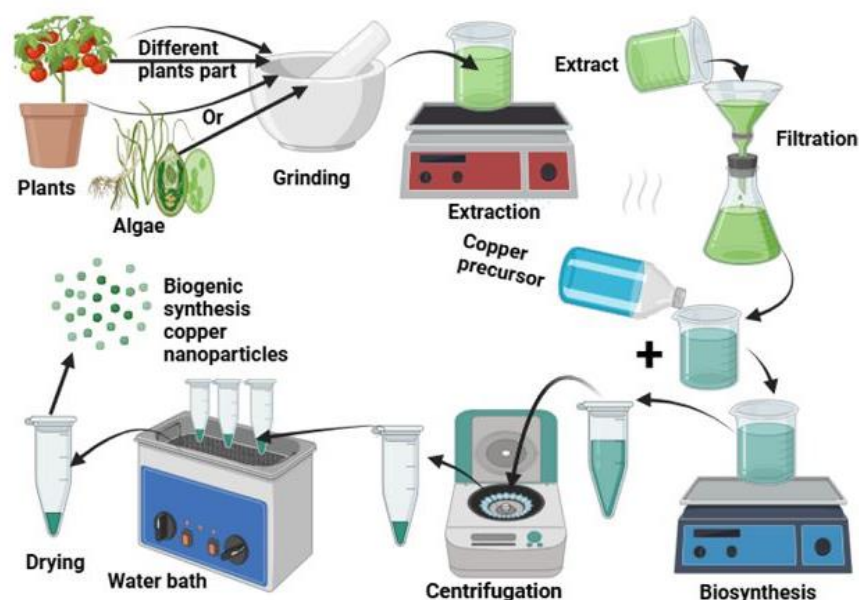


Figure 2. Process steps in the biogenic Synthesis of Copper oxide nanoparticles [Reproduced from ref. 28].

Table 2. Various Algae involve in the synthesis of CuO NPs and their Applications.

S. No.	Name of Algae	Precursor	Reaction Time	Shape	Size (in nm)	Applications	Ref.
1	<i>Macrocystis pyrifera</i>	CuSO ₄	4h	Spherical	2-50	Electronics Plant Protection	[29]
2	<i>Bifurcaria bifurcata</i>	CuSO ₄	24 h	Spherical	5-45	Fertilizer	[30]
3	<i>Cystoseria trinoids</i>	CuSO ₄	90 min	Spherical	6-7.8	Photocatalysis	[31]
4	<i>Botryococcus braunii</i>	Copper acetate	24 h	Cubical and Spherical	10-70	Antimicrobial Bio-plastics	[32]
5	<i>Anabaena cylindrica</i>	CuSO ₄	24 h	Rod-like	3.6 (inavg.)	Removing microbes from water Anticancer activity	[33]
6	<i>Sargassum polycystum</i>	-	-	Spherical	-	Antimicrobial activity	[34]
7	<i>Shewanella loihica PV-4</i>	CuCl ₂ ·2H ₂ O	24h	-	10-16	Environmental remediation Antimicrobial activities	[35]
8	<i>Sargassum longifolium</i>	CuSO ₄	24h	-	40-60	Antibacterial Antioxidant properties	[36]
9	<i>Asterarcys quadricellular e</i>	CuSO ₄	24h	Flower petals and flattened rice	4.76-13.70	Photo-catalytic activity	[37]
10	<i>Coelastrrella terrestris</i>	CuSO ₄	24h	Flower petals and flattened rice	4.26-28.51	Wastewater treatment environmental remediation's biomedical	[38]
11	<i>Spirulina platensis</i>	copper nitrate	24h	spherical shape	15.2	Photo-catalytic activity degradation of dyes environmental remediation's	[39]

Mechanistic Insight in to Algae-mediated Bio-fabrication of CuO NPs

Copper exists in multiple oxidation states, primarily Cu (I), Cu (II), and Cu(III), which influence nanoparticle formation. During green synthesis, phytochemicals present in plant extracts reduce Cu²⁺ ions to elemental Cu (Cu⁰), followed by oxidation to form CuO nanoparticles. This reduction-oxidation (redox) process is facilitated by phytochemicals such as flavonoids, phenolic compounds, tannins, terpenoids, and proteins, which act as natural reducing and stabilizing agents. The reducing potentials of the phytochemicals arise from the presence of the functional groups, which can donate electrons and

serve as efficient reducing agents. Oxygen-containing functional groups are widely recognized to play a key role in facilitating the binding of metal nanoparticles to biochemical compounds (Figure 3). The specific characteristics of the synthesized CuO NPs, such as size, shape, and dispersion, depend on variables like precursor concentration, pH, and reaction temperature.

Parameters Influencing the Algae-mediated Green Synthesis of the CuO NPs

Numerous studies have reported that the green synthesis of the algae-mediated CuO NPs is strongly influenced by the physicochemical parameters such as incubation time, temperature, pH, and

precursor concentration. These factors influence the reaction kinetics as well as the spectrophotometric characteristics of the CuO NPs.

Impact of Incubation Time:

Incubation time is a critical parameter in the algae-mediated green synthesis of the copper oxide nanoparticles, significantly influencing their formation size and properties. Proper incubation facilitates full reduction of copper ions, leading to increased nanoparticle yield, uniform nucleation, and controlled particle growth. Insufficient incubation may cause incomplete synthesis, producing smaller and less crystalline nanoparticles. Moreover, incubation time affects the optical characteristics and stability of the nanoparticles, which can be assessed using spectroscopic methods. Therefore, optimizing the incubation time is essential to obtain CuO nanoparticles with the desired structural and biological characteristics [41].

Impact of Incubation Temperature

Incubation temperature plays a vital role in the algae-mediated bio-fabrication of copper oxide nanoparticles, profoundly influencing their formation size and overall properties. Elevated temperature typically enhances the reduction of copper ions, facilitating faster nucleation and the production of smaller, more uniform nanoparticles, whereas lower

temperatures tend to slow the reaction, leading to larger or irregularly shaped particles. Temperature also impacts the crystallinity, phase development, optical behavior, and surface characteristics of the nanoparticles. Maintaining proper temperature effectively is vital for producing stable CuO nanoparticles with superior structural and biological characteristics, emphasizing its key role in optimizing green synthesis procedures [42].

Impact of Incubation pH

The incubation pH is a decisive parameter in the algae-mediated green synthesis of copper oxide nanoparticles, as it regulates the ionization state of the biomolecules and modulates the reducing ability of the phytochemicals involved in their formation. Alterations in pH have a profound impact on the nucleation, growth, and stability of the resulting nanoparticles. Alkaline conditions typically enhance the rapid reduction of the copper ions, producing small, uniformly dispersed, and stable nanoparticles, as the acidic conditions tend to slow the reduction process, leading to larger agglomerated particles. Furthermore, pH plays a direct role in determining the surface charge and colloidal stability of the CuO NPs, which in turn impact their optical, catalytic, and biological characteristics. Therefore, optimizing the incubation pH is vital to obtain CuO nanoparticles with the targeted morphology and functional performance in the green synthesis method [43].

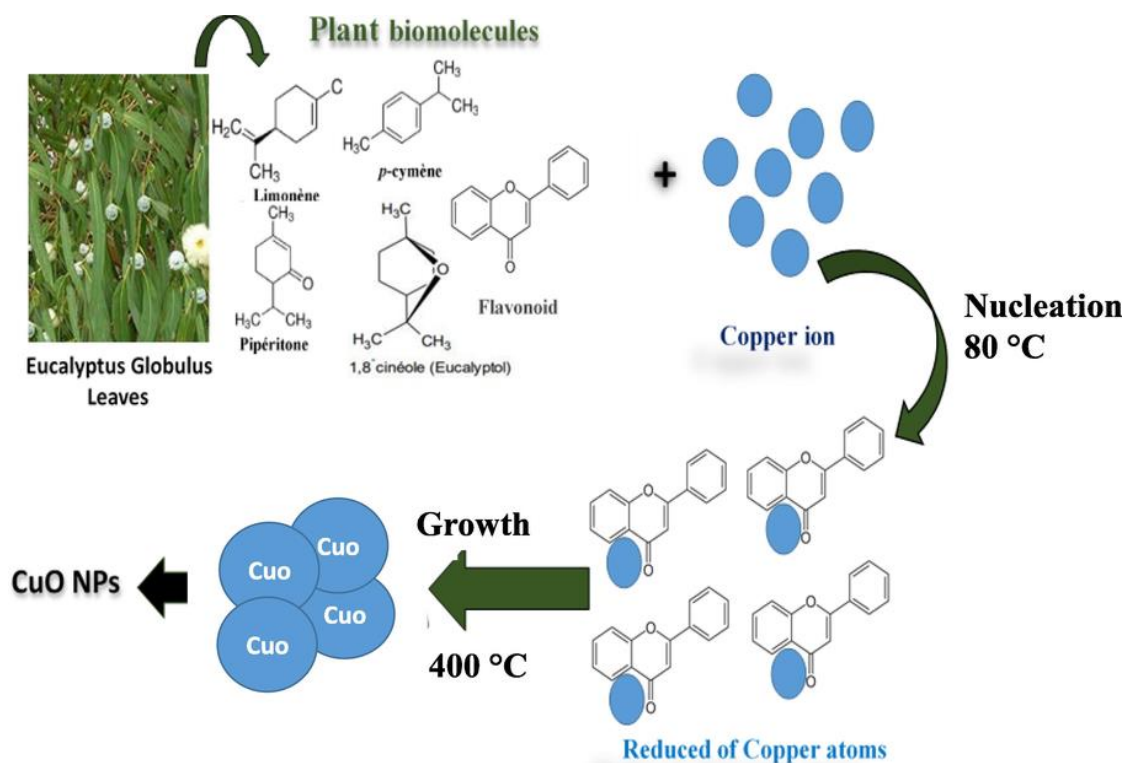


Figure 3. Mechanistic insight in to the green synthesis of Copper oxide nanoparticles [Reproduced from ref. 40].

Impact of the Precursor Concentration

The metal precursor concentration is a critical factor in the algae-mediated green synthesis of CuO nanoparticles, directly impacting nucleation, growth, and overall yield. Appropriate precursor concentrations supply enough copper ions to support uniform nucleation and controlled growth, resulting in well-dispersed and stable nanoparticles. Conversely, low precursor concentration can result in incomplete synthesis and lower yields, while very high concentration may cause particle aggregation, increased size, and polydispersity. Additionally, precursor concentration affects the surface properties, stability, and functional performance of the nanoparticles, including their optical, catalytic, and biological behavior. Therefore, precise optimization of this parameter is essential to achieve CuO nanoparticles with desired morphology stability and functionality in the green synthesis procedure [44].

Evaluation of Algae Versus Various Reducing Agents for Nanoparticles Synthesis

The biological routes of the creation of the nanoparticles offer a sustainable and eco-friendly substitute for conventional chemical and physical methods [45]. Various biological resources such as plant, algae, bacteria, and fungi have been examined as reducing and stabilizing agent although their sustainability attributes differ considerably. Plant mediated bio-fabrication utilizes readily available biomass; however, it typically require substantial plant material, which is affected by seasonal availability and involve extensive extraction procedure, which constrains scalability and increases organic waste generation. Bacterial and fungal approaches enable precise nanoparticles fabrication; however, their cultivation require sterile environment, specialized nutrient media, and stringent nutrient media, which complicate large-scale production and raise regulatory challenges due to the bio-safety concern. In contrast, algae based synthesis demonstrate significant sustainability advantages as algae require minimal resource input, proliferate rapidly using sunlight and simple nutrient, and can be cultivated in large scale in either open or closed cultivation systems with minimal environmental impact. Furthermore, algae based approaches generate comparatively lower amounts of hazardous substances and are subjected to fewer regulatory limitations since many algal species are non-pathogenic and environmentally compatible. Consequently, algae emerges a highly promising and scalable biological platform for the bio-fabrication of nanomaterials when evaluated across the key sustainability parameters (Table 2).

Plant-based Reducing Agents

Plants are commonly used in the formation of nanoparticles due to their phytochemical compositions,

including polyphenols, terpenoids, sugars, and flavonoids. These chemicals act as both reducing and stabilizing agents, helping in the formation of nanoparticles. One of the biggest advantages of using plants is their eco-friendliness and cost-effectiveness, as they do not require harmful or toxic chemicals and are easily available [46]. Also, the process happens in ambient conditions, such as temperature and pressure, making it energy-efficient as well. The main drawback of this method is that the composition of plant extract can vary, depending on the season, the location the plant was grown, and how the extract is prepared. This inconsistency often causes variation in sizes, shape, and stability of the nanoparticles. Moreover, the efficiency and yield of the plant mediated synthesis exhibit variability due to differences in metabolite composition and seasonal fluctuations in plant materials [47].

Bacteria as Reducing Agents

Bacteria are incredibly efficient in the production of nanoparticles, mainly due to their metabolic adaptability and their ability to reduce metal ions quickly. One of the key benefits of using bacteria-assisted synthesis is the level of control it provides over the morphology of the particle. With the help of genetic engineering, bacterial metabolic pathways can be modified to customize the size, shape, and surface properties of nanoparticles. This method is well-suited for large-scale or industrial production as the bacterial culture can be easily scaled up. However, bacteria need carefully controlled growth conditions, such as pH, specific temperature, and nutrient supply, which complicate the synthesis process. Another limitation is the purification of nanoparticles, as separating them from bacterial biomass requires extra purification steps. Still, bacterial-based synthesis is widely explored due to its efficiency and precision to produce well-defined nanoparticles [48].

Fungi as Reducing Agents

Fungi offer a unique benefit to nanoparticle synthesis, mainly their ability to produce particles outside the cell, making the extraction process much easier. They naturally make proteins and enzymes that aid in the reduction of metal ions and the creation of stable nanoparticles. Furthermore, fungi are more effective at creating metal-based nanoparticles because they can withstand and store higher metal concentrations. Compared to plant and bacterial-based methods, fungal synthesis provides greater control over the size and stability of the particles. Fungi tend to grow slowly, which can limit the overall production speed. However, a bigger drawback, contamination, as fungal culture requires a sterile environment to prevent the interference of unwanted microbes. Despite the limitations, fungal-mediated synthesis is considered a promising method due to its ability to produce a wide variety of nanoparticles with impressive stability [49].

Table 3. Comparison Table of different Biological Agents.

Criteria	Algae	Plants	Bacteria	Fungi
Prevalence & Diversity	Algae, including green and brown algae, are used in nanoparticle synthesis but are less explored compared to other methods.	Widely used, especially angiosperms, due to their cost-effectiveness and diverse phytochemicals.	Various bacterial species are utilized due to their high efficiency and adaptability.	Fungi are versatile biological sources with unique nanoparticle synthesis properties.
Reducing Agents	Strong binding ability toward metal ions facilitates nanoparticle formation.	Phytochemicals such as polyphenols, terpenoids, and sugars act as reducing agents.	Bacteria use intracellular and extracellular enzymes to reduce metal ions.	Fungal secreted enzymes aid in bio-reduction and nanoparticle stabilization.
Synthesis Mechanism	Can occur both intracellularly and extracellularly.	Uses phytochemicals in plant extracts to reduce metal ions into nanoparticles.	Intracellular or extracellular bio-reduction via microbial metabolism.	Primarily extracellular synthesis due to enzyme secretion.
Size & Shape Control	Moderate control, depending on species and environmental factors.	Moderate control, influenced by extract concentration, metal ions, and temperature.	High control over size and morphology through genetic engineering.	Better control over monodispersity and shape compared to bacteria and plants.
Eco-friendliness	Highly sustainable, can be cultivated in wastewater and renewable environments.	Green synthesis approach that minimizes toxic chemicals and energy use.	Environmentally benign, offering a bio-friendly alternative to chemical methods.	Considered eco-friendly, but fungal growth conditions may require optimization.
Toxicity	Lower toxicity than chemical methods, making it suitable for biomedical applications.	Generally non-toxic due to natural plant-based reducing agents.	Minimal toxicity, but requires careful selection of bacterial strains.	Low toxicity with good nanoparticle stability.
Scalability	Potential for large-scale production due to easy cultivation and rapid growth.	Scalable, but extract variability can lead to inconsistent nanoparticle properties.	High scalability, but requires optimized culture conditions for efficiency.	Scalable but slower growth rate than bacteria.
Growth Rate	Moderate; growth conditions affect synthesis speed.	Rapid biomass production, leading to fast nanoparticle synthesis.	Fast growth rate enables quick nanoparticle production.	Slower than bacteria, requiring more time for synthesis.
Purification & Extraction	Complex extraction process due to biomass entrapment of nanoparticles.	Easier extraction, but composition variability can complicate purification.	Requires additional steps to remove bacterial biomass.	Extracellular synthesis simplifies purification.
Applications	Used in water purification, bio-imaging, and antimicrobial applications.	Widely used in medicine, pharmaceuticals, and antimicrobial agents.	Applied in cancer chemotherapy, gene delivery, and biomedicine.	Used for producing nanoparticles like gold, silver, zirconia, silica, and titania.

Algae as Reducing Agents

Algae are a practical and sustainable alternative for producing nanoparticles because they are renewable

and considered to be able to build various ecosystems, even in wastewater. Biological systems, for example, yield fibre, microorganisms, and green growth, contain imperative characteristic compounds, for

example, proteins, polysaccharides, and pigments, which can serve as decreasing and balancing out operators (Table 3).

Considering old methods, the methods involving algae typically produce much more useful nanoparticles. Cysteine, apart from providing a more environmentally friendly method, had an impressive toxicity in human cells. Moreover, they have strong metal-binding properties, which also aid in efficient nanoparticle preparation [50]. The remarkable versatility they shows that they can develop in many varieties, for example, metals and metal oxides, or composite materials. In knowledge, algae as a source for nanoparticles has not been explored in-depth compared to the plant, bacteria, and fungi routes. This leaves a few gaps in our understanding of nanoparticles [51].

Advantages of Plant-mediated Synthesis

Plants are often referred to as nature's chemical factories due to their capability to generate diverse bioactive compounds. Compared to microbial synthesis, plant-assisted nanoparticle fabrication offers several advantages:

Faster Synthesis: The reaction occurs within 1–3 hours at room temperature, significantly reducing processing time.

Higher Yield & Stability: The presence of phytochemicals enhances nanoparticles' stability and dispersibility.

Safer & Energy-Efficient: Unlike bacterial or fungal synthesis, plant-based methods do not require sterile conditions, complex culturing, or prolonged incubation.

Eco-Friendly Approach: Avoids hazardous chemicals used in conventional chemical synthesis, making it a green alternative.

Due to these benefits, plant-mediated synthesis of CuO NPs has become a widely accepted method, with various plant species being explored for their ability to produce nanoparticles. The approach not only enables efficient nanoparticle fabrication but also aligns with sustainable and green chemistry principles, offering a promising route for biomedical, environmental, and industrial applications [52].

Functional Applications of the Algae-synthesized CuO NPs

Nanophycology, also known as algae-based nanotechnology, has played a vital role in the progress

of various applied scientific fields. Algal nanoparticles, due to their unique morphology and characteristics, are widely used in biosensors, environmental monitoring, pollutant remediation, water purification, and medical applications (Figure 4).

Biosensors

Algae-based green synthesized CuO NPs gained significant attention in sensor technology due to their semiconducting properties and remarkable catalytic performance. CuO nanoparticles characterized by a band gap of approximately 1.2-1.9 eV and p-type semiconducting behavior, CuO NPs exhibit high sensitivity to variation in their chemical environment, making them ideal for biosensing applications. In biosensing, CuO nanoparticles function as efficient electrode-modifying materials owing to their redox properties and excellent electron transfer capabilities. They are frequently employed in electrochemical biosensors for the detection of biologically significant molecules, including cholesterol, dopamine, uric acid, glucose, and hydrogen peroxide. Algae-mediated green synthesized CuO nanoparticles enhance their biocompatibility and mitigate the toxicity associated typically linked to the chemically synthesized counterpart which is essential for applications in the biological system [53]. The surface bound algal bio-molecules like polysaccharides, phenolic compounds and protein function as natural capping and stabilizing layer, facilitating improved surface reactivity. The biocapping layer contributes to enhanced biocompatibility and minimizing in vitro cytotoxicity, which is a critical requirement for biosensor designed for biological applications.

Nano-fertilizers

Algae-based green method synthesized CuO NPs have shown significant potential as a nano-fertilizer by delivering essential micronutrients, especially copper, in a controlled and efficient way. Copper serves as an essential micronutrient and plays a key role in various physiological processes, including respiration, photosynthesis, and the activity of multiple enzymes. Conventional copper fertilizers exhibit low bioavailability and are susceptible to rapid leaching, resulting in nutrient loss and potential environmental contamination. Conversely, CuO NPs, with their nanoscale dimension and high surface area, enable controlled and target release of copper ions, enhancing nutrient uptake by plant roots and minimizing the potential toxicity [54]. Algae assisted synthesized copper oxide nanoparticles exhibit reduced phototoxicity and improved plant compatibility relative to the chemically synthesized counterpart, owing to their bio-functionalized surface and the absence of harsh chemical stabilizing agent.

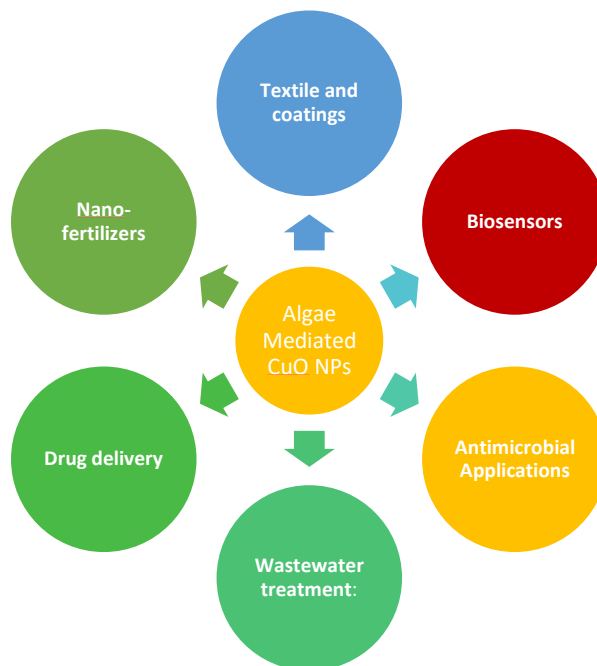


Figure 4. Potential Applications of the algae mediated CuO nanoparticles.

Textile and Coatings

Algae-based green synthesized copper oxide nanoparticles have drawn significant attention in the textile and coating sector due to their potent antimicrobial and antifouling capabilities. When incorporated into fabric or surface coating these nanoparticles offer durable defense against bacterial fungi and other microbial agents, thereby minimizing the risk of infections and controlling unpleasant odor in clothing and medical textiles. Their nanoscale dimension ensures even distribution within the fiber, enhancing surface interaction with microorganisms and boosting antimicrobial efficacy [55].

Drug Delivery

Algae-mediated green-synthesized copper oxide nanoparticles demonstrated considerable potential as a nanocarrier for drug delivery owing to their distinctive physicochemical properties. These nanoparticles, through encapsulation of therapeutic agents, facilitate controlled and site-specific drug release, enhancing treatment efficacy while reducing adverse effects. The bioactive compounds present on the surface of the developed nanoparticles not only improve biocompatibility but also facilitate selective interactions with the target cells such as bacterial cells, cancer cells, or specific tissue. In addition, CuO nanoparticles

are capable of responding to stimuli like pH, temperature or redox conditions, enabling controlled drug release and making them ideal for targeted, specific therapeutic applications. Overall, the synergy of the green synthesis and enhanced biocompatibility makes CuO NPs a promising platform for advanced drug delivery applications in biomedicine [56].

Wastewater Treatment

Algae-mediated green synthesized copper oxide nanoparticles have been extensively explored for waste water photocatalysis driven by their advantageous electronic and optical properties. With a relative narrow band gap, CuO NPs allow efficient absorption of solar and UV light, facilitating the formation of a reactive electron-hole pair. These charge carriers interact with water and oxygen molecules to form reactive oxygen species, including hydroxyl free radical and superoxide radical anion, which can efficiently oxidize and degrade a variety of organic pollutant including dye, pharmaceutical and industrial waste. The large surface to volume ratio of copper oxide nanoparticles, combined with natural stabilizing and capping agents from the algal extract, promotes the adsorption of contaminants onto their surface, thereby enhancing degradation efficiency [57].

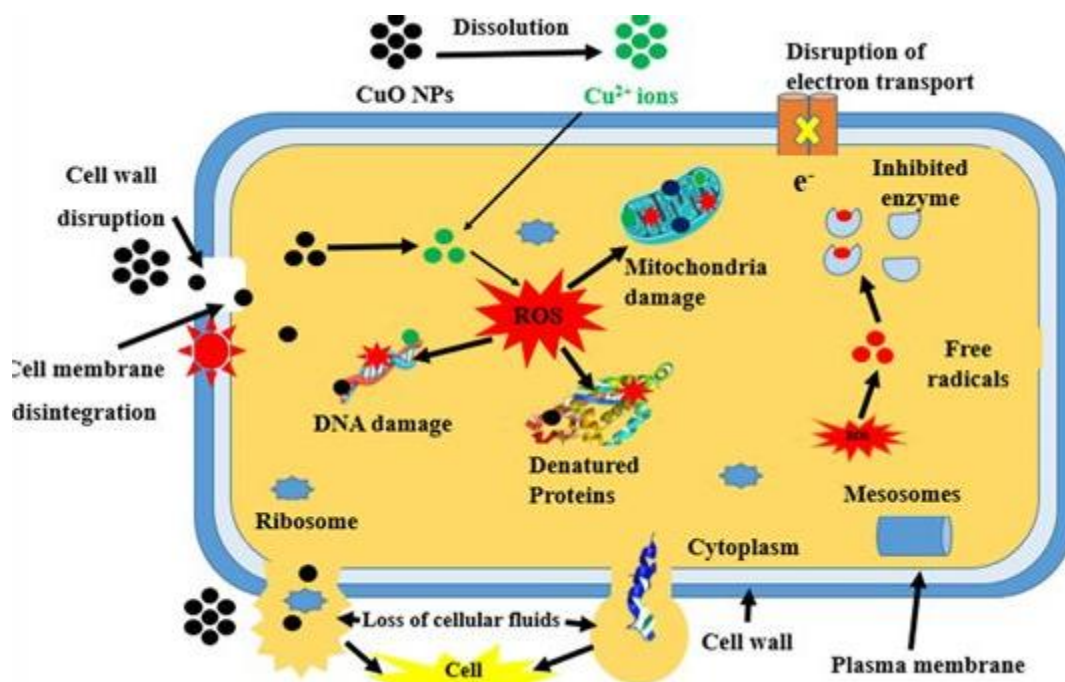


Figure 5. Mode of antimicrobial action of the copper oxide nanoparticles [Reproduced from ref. 59].

Antimicrobial Applications

Algae-mediated green-synthesized CuO NPs exhibit significant antimicrobial potential against a wide range of microorganisms, including Gram-positive and Gram-negative bacteria, fungi and certain viruses. Their efficiency is mainly driven by several mechanism including the generation of reactive oxygen species, which induce oxidative damage to lipids, protein and nucleic acids; disruption of cell membrane leading to leakage of intercellular content, and the release of copper ion which disrupt enzymatic activity and impair DNA replications (Figure 5). Furthermore these nanoparticles inhibit microbial adhesion and biofilm development, enhancing their effectiveness against persistent and drug resistant pathogen. In comparison to chemically synthesized CuO NPs, algae-mediated CuO NPs demonstrate improved biocompatibility and stability due to the natural capping agent from algal metabolites, while also reducing cytotoxic effects. The enhance antimicrobial activity arises from bio-capped nanoparticles surface formed by algal metabolites enabling more effective interactions with microbial membrane and regulated generation of the reactive oxygen species [58].

Toxicological Impacts of the CuO Nanoparticles

Copper oxide nanoparticles are toxic to both the human in vitro and in vivo systems, and they also affect several animals. Studies have shown that bioavailability plays a key role in their toxicity, similar to other hazardous metals. Several factors influence CuO-NP toxicity.

Size & Morphology: Smaller nanomaterials tend to be more toxic than their larger counterpart.

Surface Charge: Positively charged NPs increase cellular interactions and toxicity.

Dissolution: The breakdown of CuO NPs depends on pH and temperature, impacting toxicity. CuO-NPs have been found to be 20 times more toxic to the protozoa *Tetrahymena thermophila* than their bulk counterpart. Toxicity also depends on exposure duration, peaking between 4–24 hours. Studies on algae (*Chlamydomonas reinhardtii*) demonstrated that CuO NPs were more harmful than their bulk form, with toxicity persisting for 72 hours. Their dissolution in simulated freshwater further enhances toxicity [60]. Animal studies have shown CuO NPs to be lethal to *Xenopus laevis* larvae, causing 40% mortality after 120 hours, and highly toxic to *Cyprinus carpio* fish. Their toxicity mechanism is linked to mitochondrial damage, DNA fragmentation, and oxidative stress. In vitro research revealed that CuO NPs impair cell proliferation and are particularly toxic to specific cell types. ROS generation, DNA damage, and reduced glutathione (GSH) depletion contribute to cytotoxicity. CuO NPs exhibit greater toxicity in human lung and skin cells compared to micro-sized particles. Studies indicate oxidative stress induction in various cell lines, leading to membrane damage, hemolysis, and increased genotoxicity. Research on *Capitella teleta* worms found that CuO NPs caused a 10% reduction in survival, impacting uptake and depuration rates. Investigations on respiratory epithelial cells confirmed that CuO-NPs induce oxidative stress and

cytotoxicity, even at low doses. Additionally, CuO NPs have been linked to melanoma cell growth but also show potential in treating triple-negative breast cancer when synthesized with folic acid [61]. Neuronal studies suggest CuO NPs compromise cell membranes via a Trojan horse mechanism, penetrating cells, dissolving in acidic environments, and releasing toxic metal ions. These findings highlight the need for further research into CuO NPs' toxicity mechanisms and safe applications.

Future Directions and Applications of the CuO Nanoparticles

Algae-mediated green synthesis of the copper oxide nanoparticles provides a sustainable, eco-friendly, and cost-effective approach with promising potential for future applications. This approach demonstrates potential in biomedical applications such as anticancer, antimicrobial, and targeted drug delivery owing to the biocompatible nature of the nanoparticles. From the environmental perspective, these nanoparticles serve applications in wastewater treatment, degradation of pollutants, and heavy metal removal. Moreover, their catalytic and photocatalytic properties render them promising candidates for applications in chemical analysis and energy storage applications. Future studies are anticipated to focus on creating a nanocomposite, advancing scalable synthesis protocols for industrial applications, and clarifying of molecular mechanisms involved in the nanoparticles synthesis. Enhancing these factors will improve the functional performance, reliability, and commercial potential of mediated CuO nanoparticles, emphasizing their wide range of applications in medicine, environmental remediation, and nanotechnology [62].

CONCLUSION

To summarize, because of nanomaterials' special qualities and wide range of uses, they have enormous potential in a number of disciplines, such as environmental science, electronics, medicine, and energy. Nanoscale matter manipulation has created new opportunities for creativity and technical development. Although copper oxide nanoparticles (CuO NPs) have many uses, traditional synthesis's sustainability problems have prompted a significant move toward more environmentally friendly alternatives. Green synthesis techniques provide environmentally acceptable and sustainable ways to produce nanoparticles by using biological agents like algae. The responsible and efficient use of nanomaterials to address global issues and enhance the quality of life is being made possible by continuous research and development activities, even though there are still issues with large-scale production, safety evaluations, and standardization.

Declarations

Ethical Approval

Not Applicable

Competing Interests

No any kind of competing interest in this work.

Data Availability

The data made available on reasonable request.

Funding

Not Applicable

Author Contribution Statement

Disha Agarwal: Formal analysis, Writing - Original Draft

Shreyoshi Sarkar: Formal analysis, Writing - Original Draft

Poonam Negi: Writing - Review & Editing, Resources.

Harish Chandra Joshi: Writing - Review & Editing, Visualization.

Waseem Ahmad: Writing - Review & Editing, Conceptualization, Supervision.

Shalini Upadhyay: Writing - Review & Editing, Visualization.

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