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Eco-friendly Cobalt Oxide Nanoparticles for the Control of Harmful Cyanobacterial Bloom (*Oscillatoria* sp.) in Aquatic Systems

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Abstract

Harmful cyanobacterial blooms have become a significant environmental concern in freshwater ecosystems due to their adverse effects on water quality, aquatic biodiversity, and public health. The excessive proliferation of cyanobacteria such as *Oscillatoria* sp. leads to oxygen depletion, toxin production, and deterioration of aquatic environments. In the present study, cobalt oxide (Co_3O_4) nanoparticles were synthesized through an eco-friendly green synthesis approach using ginger extract as a reducing and stabilizing agent. The synthesized nanoparticles were characterized using various physicochemical techniques to confirm their functional group and morphological properties. The anti-blooming potential of Co_3O_4 nanoparticles was evaluated against the cyanobacterium *Oscillatoria* sp. by assessing growth parameters including chlorophyll content and growth rate under different nanoparticle concentrations. The results revealed a significant concentration-dependent inhibition of cyanobacterial growth. Exposure to Co_3O_4 nanoparticles caused a marked reduction in chlorophyll pigments and biomass productivity, indicating disruption of photosynthetic activity and cellular metabolism. The inhibitory effects are likely associated with nanoparticle–cell interactions that impair physiological processes in cyanobacterial cells. These findings demonstrate that green synthesized Co_3O_4 nanoparticles can effectively suppress cyanobacterial proliferation and may serve as a promising eco-friendly strategy for controlling harmful algal blooms in aquatic systems and improving water quality.

Key Words: Green synthesis, Cobalt oxide nanoparticles, Cyanobacterial bloom control, *Oscillatoria* sp., Anti-blooming activity, Aquatic ecosystem management

Introduction

Harmful cyanobacterial blooms have become a serious environmental problem in freshwater ecosystems worldwide due to increasing nutrient enrichment and climate change. Excessive inputs of nutrients such as nitrogen and phosphorus from agricultural runoff, domestic wastewater, and industrial discharges stimulate the rapid proliferation of cyanobacteria, resulting in dense surface blooms that disrupt aquatic ecosystems [1]. These blooms significantly reduce light penetration, alter food web structures, and deplete dissolved oxygen levels in water bodies. In addition, many cyanobacteria produce toxic secondary metabolites known as cyanotoxins, which pose severe risks to aquatic organisms, livestock, and human health. Consequently, the occurrence of cyanobacterial blooms has become a major challenge for water resource management and ecosystem sustainability [3,5]. Among the various bloom-forming cyanobacteria, *Oscillatoria* sp. is widely distributed in freshwater environments and is known for its rapid growth and high adaptability to nutrient-rich conditions. The excessive proliferation of *Oscillatoria* can lead to significant ecological disturbances, including reduced biodiversity and deterioration of water quality. Furthermore, the presence of cyanobacterial blooms in drinking water sources creates serious public health concerns due to the potential release of harmful metabolites. Therefore, effective and environmentally sustainable strategies are required to control the growth of bloom-forming cyanobacteria and to restore the ecological balance of aquatic systems [8]. Several conventional methods have been employed to control cyanobacterial blooms, including chemical algaecides, biological control agents, and physical removal techniques. Chemical treatments such as copper sulfate are commonly used for algal control; however, their long-term application may result in secondary pollution, toxicity to non-target organisms, and accumulation of heavy metals in aquatic environments. Biological approaches using algicidal bacteria and grazing organisms have also been investigated, but their effectiveness is often limited by environmental variability and ecological complexity. Similarly, physical methods such as aeration, filtration, and sediment dredging are generally expensive and may not provide long-term solutions for bloom mitigation. These limitations highlight the need for alternative strategies that are both efficient and environmentally benign.In recent years, nanotechnology has emerged as a promising approach for addressing environmental challenges, including water pollution and harmful algal bloom control. Nanomaterials possess unique physicochemical properties such as high surface area, enhanced reactivity, and strong interaction with microbial cells, which make them effective agents for environmental remediation [12]. Various metal and metal oxide nanoparticles have been explored for their antimicrobial and algicidal properties. These nanoparticles can interact with microbial cells through multiple mechanisms, including membrane disruption, generation of reactive oxygen species (ROS), inhibition of photosynthesis, and interference with cellular metabolic pathways. Such properties make nanomaterials potential candidates for controlling harmful cyanobacterial growth in aquatic systems [17]. Among different metal oxide nanomaterials, cobalt oxide (Co_3O_4) nanoparticles have attracted increasing attention due to their excellent catalytic activity, stability, and environmental applicability. Co_3O_4 nanoparticles exhibit strong oxidative potential and surface reactivity, which can significantly affect microbial cell viability. Previous studies have reported that cobalt-based nanomaterials possess antimicrobial properties against various microorganisms. These nanoparticles can induce oxidative stress in microbial cells through the production of reactive oxygen species, leading to damage of cellular components such as proteins, lipids, and nucleic acids. In addition, the high surface area of Co_3O_4 nanoparticles facilitates strong interaction with cell membranes, potentially disrupting cellular integrity and physiological processes. Recently, green synthesis approaches have been widely adopted for nanoparticle production to reduce the environmental and health hazards associated with conventional chemical synthesis methods. Plant extracts contain various bioactive compounds such as phenolics, flavonoids, and proteins that can act as natural reducing and stabilizing agents during nanoparticle formation [21]. Green synthesis not only provides an environmentally friendly and cost-effective method for nanoparticle production but also enhances the biocompatibility and stability of the resulting nanomaterials. Therefore, phytogenic nanoparticles are increasingly being explored for environmental remediation applications.Despite the growing interest in nanotechnology for environmental management, studies focusing on the use of cobalt oxide nanoparticles for controlling cyanobacterial blooms remain limited. In particular, the inhibitory effects of Co_3O_4 nanoparticles on bloom-forming cyanobacteria such as *Oscillatoria* sp. have not been extensively investigated. Understanding the interaction between nanoparticles and cyanobacterial cells is essential for developing effective anti-blooming strategies that minimize ecological risks. Therefore, the present study aims to synthesize eco-friendly cobalt oxide (Co_3O_4) nanoparticles through a green synthesis approach and evaluate their anti-blooming potential against *Oscillatoria* sp. The effects of Co_3O_4 nanoparticles on cyanobacterial growth were assessed by analyzing key physiological parameters including chlorophyll content, biomass accumulation, and growth rate. The findings of this study provide valuable insights into the potential application of green synthesized cobalt oxide nanoparticles as an effective and sustainable strategy for controlling harmful cyanobacterial blooms in aquatic environments.

Experimental

Collection and Cultivation of Cyanobacteria: The cyanobacterial species *Oscillatoria* sp. was collected from a freshwater tank and maintained under laboratory conditions. The culture was grown in sterilized BG-11 medium and incubated under controlled environmental conditions with a temperature of 25 ± 2 °C and a 12 h light/12 h dark photoperiod. Illumination was provided using cool white fluorescent lamps with an intensity of approximately 3000–4000 lux. The cultures were periodically subcultured to maintain active growth and to ensure the availability of healthy cells for experimental analysis.

Preparation of ginger extract: Fresh ginger (*Zingiber officinale*) rhizomes were thoroughly washed with distilled water to remove adhering impurities and surface contaminants. The cleaned rhizomes were then **chopped into small pieces and crushed using a mortar and pestle.** The resulting pulp was **filtered through Whatman No. 1 filter paper**, and the obtained filtrate was collected as a **concentrated ginger extract**. The extract was stored at **4 °C** and can used freshly as a **reducing and stabilizing agent** for the green synthesis of cobalt oxide nanoparticles.

Green synthesis of Cobalt Oxide nanoparticles: Cobalt oxide nanoparticles were synthesized via a green route using the prepared ginger extract as a bio reductant and capping agent. A 0.1 M aqueous solution of cobalt chloride hexahydrate was prepared in 50 mL of distilled water and stirred magnetically at room temperature. To this solution, 10 mL of freshly prepared ginger extract was added dropwise under constant stirring. The mixture was stirred for 2 h at ambient temperature, during which the colour gradually changed from pinkish to dark brown, confirming the initiation of nanoparticle formation. The reaction mixture was allowed to stand undisturbed for 24 h to complete the reduction process. The resulting precipitate was centrifuged at 8000 rpm for 15 min, and the collected pellet was washed repeatedly with distilled water and ethanol to remove unreacted species and organic residues. The purified nanoparticles were oven-dried at 80 °C for 6 h and subsequently calcined at 400 °C for 3 h in a muffle furnace to obtain phase-pure crystalline Co_3O_4 NPs suitable for characterization and biological evaluation.

Anti-Blooming activity: The anti-blooming activity of cobalt oxide nanoparticles was evaluated by exposing *Oscillatoria* sp. cultures to different nanoparticle concentrations. Actively growing cyanobacterial cultures were transferred into sterile conical flasks containing BG-11 medium. Various concentrations of Co_3O_4 nanoparticles (for example 10, 20, 30, 40, and 50 µg mL^{-1}) were added to the experimental cultures, while a culture without nanoparticles served as the control. All experimental setups were maintained under the same incubation conditions as described for the culture maintenance. The experiments were conducted for a specified period, and samples were periodically collected to evaluate the effects of nanoparticles on cyanobacterial growth.

Characterization of Cobalt Oxide Nanoparticles: The synthesized cobalt oxide nanoparticles were characterized using various analytical techniques to determine their structural and physicochemical properties. Fourier Transform Infrared Spectroscopy (FTIR) was performed to identify functional groups involved in nanoparticle stabilization. Transmission electron microscopy (TEM) provided information on particle morphology, size, and dispersion. These characterization techniques confirmed the successful synthesis of cobalt oxide nanoparticles.

Results and Discussion

Fourier Transform Infrared Spectroscopy (FTIR): A broad band at ~3426 cm^{-1} corresponds to O–H stretching vibrations, indicating hydroxyl groups from water or phenolic compounds that likely contributed to nanoparticle reduction and stabilization. Peaks at ~2911 cm^{-1} and ~1638 cm^{-1} are attributed to C–H and C=O stretching vibrations, suggesting aldehyde or ketone groups in the extract. The absorption at ~1422 cm^{-1} corresponds to C–C stretching of aromatic compounds, while the peak at ~1056 cm^{-1} indicates C–O stretching of primary alcohols. Additionally, bands at ~504 cm^{-1} and ~664 cm^{-1} are characteristic of Co–O stretching vibrations, confirming the formation of spinel-structured Co_3O_4 nanoparticles. These observations collectively demonstrate that phytochemicals in the ginger extract acted as both reducing and stabilizing agents during the green synthesis process.

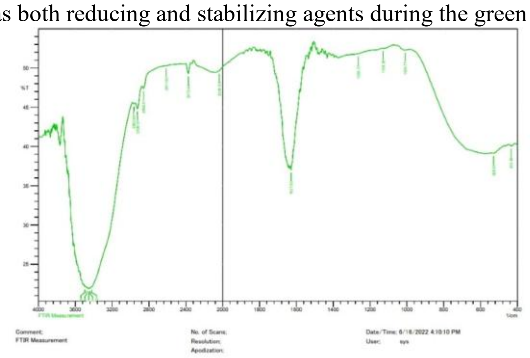

Fig. 1 FTIR Spectrum of green synthesized cobalt oxide nanoparticles

Transmission electron microscopy (TEM)

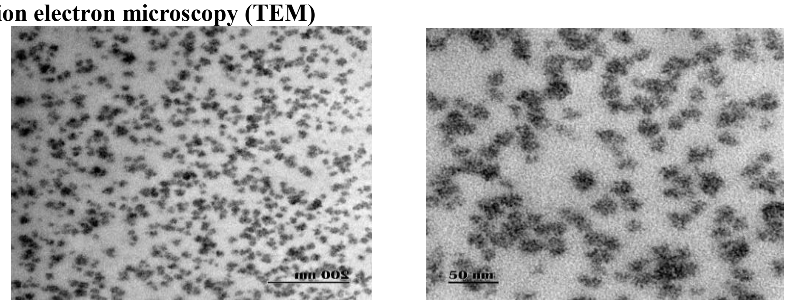

Fig. 2 TEM image of Cobalt oxide nanoparticles using ginger extract

TEM analysis demonstrated that the Co_3O_4 nanoparticles were predominantly spherical, with a size range of approximately 15–25 nm. Minimal aggregation was observed, indicating effective stabilization by ginger phytochemicals. The nanoscale size and uniform morphology enhance surface interactions with bacterial cells, supporting their anti-blooming efficacy. Compared to previously reported Co_3O_4 nanoparticles synthesized using other plant extracts or chemical routes, the ginger-mediated approach achieved **enhanced bioactivity** due to synergistic interaction between bioactive phytochemicals and metal oxide surfaces, promoting **ROS generation and EPS degradation**.

Growth Inhibition of *Oscillatoria* sp. by Co_3O_4 Nanoparticles

The anti-blooming activity of the synthesized cobalt oxide (Co_3O_4) nanoparticles was evaluated by monitoring the growth response of *Oscillatoria* sp. under different nanoparticle concentrations. The results clearly demonstrated that exposure to Co_3O_4 nanoparticles significantly suppressed the growth of the cyanobacterium compared to the untreated control culture. A concentration-dependent inhibitory effect was observed, where increasing nanoparticle concentrations resulted in greater suppression of cyanobacterial growth. At lower nanoparticle concentrations, a moderate reduction in growth was observed, suggesting partial interference with cellular processes. However, at higher concentrations, the growth of *Oscillatoria* sp. was markedly reduced, indicating that the nanoparticles effectively limit cyanobacterial proliferation. The observed inhibition may be attributed to the interaction between the nanoparticles and the cyanobacterial cells, which can affect cell membrane integrity and disrupt normal metabolic functions.

Table. 1 Effect of nanostructured Co_3O_4 on the specific growth rate of *Oscillatoria* sp.

Conc. of Co_3O_4 NPs (mg/50mL)	Specific Growth Rate (µ)
Control	3.15
0.652	2.71
1.25	2.76
2.5	2.54
5	1.91
10	0.43

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The high surface area and reactive nature of cobalt oxide nanoparticles facilitate their adsorption onto the surface of cyanobacterial cells. Such interactions can lead to structural damage to the cell membrane and increased membrane permeability, resulting in leakage of intracellular components. In addition, cobalt oxide nanoparticles are known to induce oxidative stress through the generation of reactive oxygen species (ROS). These reactive species can damage essential cellular components including proteins, lipids, and nucleic acids, ultimately impairing cell viability and growth. Furthermore, nanoparticle exposure may interfere with photosynthetic processes in cyanobacteria. Since photosynthesis is the primary mechanism through which cyanobacteria obtain energy for growth, any disruption in photosynthetic activity can significantly reduce cellular productivity and biomass accumulation. The combined effects of membrane disruption, oxidative stress, and photosynthetic inhibition contribute to the observed reduction in the growth of *Oscillatoria* sp.

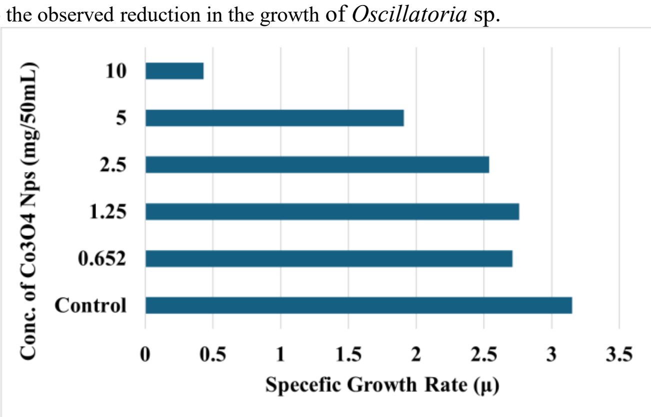

Fig. 3 Growth rate response of *Oscillatoria* sp. to Co_3O_4 treatment

The highest concentration (10 mg/50 mL) caused a drastic reduction of µ to 0.43, corresponding to 86.35% inhibition relative to the control. The results obtained in the present study suggest that cobalt oxide nanoparticles possess strong inhibitory effects against bloom-forming cyanobacteria. The concentration-dependent suppression of cyanobacterial growth indicates that these nanoparticles can effectively limit excessive proliferation of *Oscillatoria* sp., thereby offering a promising strategy for controlling harmful cyanobacterial blooms in aquatic environments. Therefore, the application of green synthesized Co_3O_4 nanoparticles may provide an efficient and environmentally friendly approach for bloom mitigation and water quality management.

Chlorophyll pigment suppression

Chlorophyll pigments play a crucial role in the photosynthetic activity of cyanobacteria and are widely used as indicators of algal growth and physiological status. In the present study, the effect of cobalt oxide (Co_3O_4) nanoparticles on the chlorophyll content of *Oscillatoria* sp. was investigated to evaluate the impact of nanoparticle exposure on photosynthetic performance. The results revealed a noticeable reduction in chlorophyll concentration in the nanoparticle-treated cultures compared to the untreated control. The control culture exhibited higher chlorophyll levels, indicating normal photosynthetic activity and active cyanobacterial growth. However, upon exposure to different concentrations of Co_3O_4 nanoparticles, a gradual decline in chlorophyll content was observed. The decrease in chlorophyll pigments became more pronounced with increasing nanoparticle concentration, indicating a clear concentration-dependent inhibitory effect on the photosynthetic machinery of *Oscillatoria* sp. This reduction suggests that cobalt oxide nanoparticles interfere with pigment synthesis and disrupt the normal functioning of photosystems.

Table. 2 Chlorophyll content of *Oscillatoria* sp. following Co_3O_4 nanoparticle exposure

Conc. of Co_3O_4 NPs (mg/50mL)	Chlorophyll (mg/L)
Control	39.86
0.652	37.38
1.25	28.4
2.5	24.63
5	19.11
10	12.01

The suppression of chlorophyll pigments may be attributed to several mechanisms associated with nanoparticle–cell interactions. Cobalt oxide nanoparticles can attach to the surface of cyanobacterial cells due to their high surface reactivity and nanoscale dimensions. Such interactions may lead to structural damage to the cell membrane and internal cellular components. Furthermore, nanoparticles are known to generate reactive oxygen species (ROS), which can induce oxidative stress within cyanobacterial cells. Excessive ROS production can damage chloroplast structures, degrade photosynthetic pigments, and impair electron transport processes involved in photosynthesis. Another possible explanation for the reduction in chlorophyll content is the disruption of pigment biosynthesis pathways. Nanoparticle exposure may interfere with enzymatic activities involved in chlorophyll formation, thereby reducing pigment accumulation in cyanobacterial cells. As chlorophyll pigments are essential for capturing light energy and driving photosynthetic reactions, their degradation directly affects the energy metabolism and growth potential of the organism. Another possible explanation for the reduction in chlorophyll content is the disruption of pigment biosynthesis pathways. Nanoparticle exposure may interfere with enzymatic activities involved in chlorophyll formation, thereby reducing pigment accumulation in cyanobacterial cells. As chlorophyll pigments are essential for capturing light energy and driving photosynthetic reactions, their degradation directly affects the energy metabolism and growth potential of the organism.

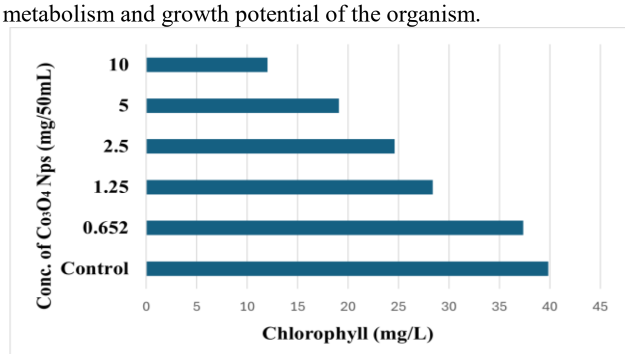

Fig. 4 Chlorophyll pigment suppression of *Oscillatoria* sp. by Co_3O_4 Nanoparticles

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The highest concentration resulted in a 69.88% reduction relative to the control, indicating substantial impairment of the photosynthetic apparatus. The observed decline in chlorophyll concentration clearly indicates that Co_3O_4 nanoparticles exert a strong inhibitory effect on the photosynthetic system of *Oscillatoria* sp. The impairment of photosynthetic activity ultimately leads to reduced cellular productivity and suppression of cyanobacterial proliferation. These findings demonstrate that cobalt oxide nanoparticles can effectively inhibit the photosynthetic efficiency of bloom-forming cyanobacteria and may serve as a promising anti-blooming agent for controlling harmful cyanobacterial growth in aquatic environments.

Integrated Anti-Blooming Mechanism of Co_3O_4 Nanoparticles against *Oscillatoria* sp.

Based on the experimental observations obtained in the present study, a possible mechanism for the anti-blooming activity of cobalt oxide (Co_3O_4) nanoparticles against *Oscillatoria* sp. can be proposed. The inhibition of cyanobacterial growth appears to be the result of multiple interactions between the nanoparticles and cyanobacterial cells, leading to physiological and structural disturbances that ultimately suppress bloom formation. One of the primary mechanisms involves the **adsorption of nanoparticles onto the cyanobacterial cell surface**. Due to their nanoscale size and high surface reactivity, Co_3O_4 nanoparticles can easily interact with the extracellular surface of *Oscillatoria* cells. This interaction may lead to **physical damage to the cell membrane**, altering membrane permeability and disrupting the normal exchange of nutrients and metabolites. Such structural disturbances can impair cellular homeostasis and reduce cell viability. Another important mechanism is the **generation of reactive oxygen species (ROS)** in the presence of nanoparticles. Metal oxide nanoparticles are known to produce ROS such as hydroxyl radicals and superoxide ions under environmental conditions. Excessive accumulation of these reactive species can induce **oxidative stress within cyanobacterial cells**, leading to damage of essential cellular components including proteins, lipids, and nucleic acids. Oxidative stress can also affect enzyme activities involved in cellular metabolism and growth.

Furthermore, the present study demonstrated a **significant reduction in chlorophyll pigments**, indicating that Co_3O_4 nanoparticles interfere with the photosynthetic system of *Oscillatoria* sp. The degradation of chlorophyll pigments and disruption of the photosynthetic apparatus may reduce the efficiency of light energy absorption and electron transport processes. As photosynthesis is the primary source of energy for cyanobacterial growth, impairment of this process directly limits cellular productivity and proliferation. The combined effects of **membrane damage, oxidative stress, and photosynthetic inhibition** ultimately result in a decline in growth rate and biomass accumulation of *Oscillatoria* sp. These processes collectively contribute to the suppression of cyanobacterial growth and prevent the excessive proliferation responsible for harmful algal blooms. Therefore, the green synthesized cobalt oxide nanoparticles demonstrate a strong **anti-blooming effect through multiple inhibitory pathways**. The ability of Co_3O_4 nanoparticles to disrupt cyanobacterial physiology suggests their potential application as an eco-friendly strategy for controlling harmful cyanobacterial blooms and improving water quality in aquatic ecosystems.

Conclusion

The present study demonstrated the successful synthesis of cobalt oxide (Co_3O_4) nanoparticles through a green synthesis approach and evaluated their potential for controlling harmful cyanobacterial growth. Characterization analyses confirmed the formation of crystalline and nanoscale Co_3O_4 particles with suitable physicochemical properties. The biological evaluation revealed that the synthesized nanoparticles exhibited significant inhibitory effects against *Oscillatoria* sp. The reduction in chlorophyll pigments and the decline in growth rate clearly indicated that nanoparticle exposure disrupted the photosynthetic efficiency and metabolic activity of the cyanobacterium. The anti-blooming activity of Co_3O_4 nanoparticles is likely associated with multiple mechanisms including membrane damage, oxidative stress, and interference with photosynthetic processes. These combined effects effectively suppressed cyanobacterial proliferation. Therefore, green synthesized cobalt oxide nanoparticles show promising potential as an eco-friendly strategy for controlling harmful cyanobacterial blooms and improving water quality in aquatic ecosystems.

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