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RESEARCH ARTICLE

Use of Iron Nanoparticles to remove waterborne pathogens

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ABSTRACT

In recent sciences Nanotechnology is a burning field for the researchers. Nanotechnology deals with the Nanoparticles having a size of 1-100nm in one dimension used significantly concerning medical chemistry, atomic physics, and all other known fields. Nanoparticles are ultrafine materials with unique properties due to their small size and high surface area. These properties make them highly effective in a variety of applications, including water treatment. Waterborne pathogens are a major cause of water pollution, contributing to numerous public health issues worldwide. Contaminated water sources, such as rivers, ponds, and sewage systems, often harbour harmful microorganisms that pose significant risks to human health. Among the various types of nanoparticles, iron nanoparticles have shown considerable promise in the removal of waterborne pathogens. This approach involves the use of iron nanoparticles for the removal of waterborne pathogens was investigated in this study to assess their potential in water purification. Green synthesis of iron nanoparticles using nitric acid and ferric chloride was done. Water sample—sewage water—were collected for analysis. Total viable count (TVC) was performed on the untreated sample, and the sample were subsequently treated with iron nanoparticles to evaluate their pathogen removal efficacy. A filter was constructed incorporating the synthesized nanoparticle. The results demonstrated a significant reduction in microbial load in all water samples, indicating that the synthesized nanoparticles effectively aided in pathogen removal and can be considered as viable candidates for water purification applications.

Keywords: Antimicrobial, Carbon, Iron nanoparticle, Nanotechnology, Nano-based.

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INTRODUCTION

Nanotechnology and Nanoparticles: An Overview: Nanotechnology involves manipulating materials at the nanoscale (1–100 nm), where matter exhibits unique physical, chemical, and biological properties (Kumari and Sarkar, 2021). A core component of this field is nanoparticles (NPs), which range from 10 to 1000 nm and can be synthesized from proteins, polysaccharides, lipids, and metals (Mohanraj and Chen, 2006). Structurally, they often include a surface layer, shell, and core (Khan et al., 2019). Their high surface area-to-volume ratio, reactivity, optical behavior, and magnetic or electrical conductivity allow broad application across fields such as medicine, agriculture, and environmental engineering (Moustafa, 2017).

Nanoparticles in Environmental Applications: NPs are increasingly used in water treatment to remove heavy metals, organic pollutants, and pathogens. Their portability and low cost make them suitable for remote settings. Nanoparticles are also used for air purification, waste treatment, and agricultural enhancement through controlled pesticide/fertilizer release and soil remediation. Additional applications span electronics, textiles, cosmetics, pharmaceuticals, and food packaging (Kumari and Sarkar, 2021). Antimicrobial Mechanisms of Nanoparticles: NPs exert antimicrobial effects through multiple mechanisms, including: 1) Membrane Disruption – Sharp-edged NPs penetrate microbial membranes, causing leakage and cell death. 2) Reactive Oxygen Species (ROS) Generation – ROS like hydroxyl radicals damage microbial DNA, proteins, and lipids. 3) Metabolic Interference – NPs impair key microbial enzymatic functions. 4. Biofilm Disruption – NPs inhibit or degrade microbial biofilms, which are difficult to remove by conventional methods. 5. Ion Release – Metal-based NPs (e.g., silver, copper) release toxic ions that deactivate microbial enzymes and DNA (Olawale et al., 2024). Types of Nanoparticles: 1) Organic Nanoparticles: Made from biodegradable polymers and lipids, examples include dendrimers, liposomes, and micelles. They are widely used in drug delivery due to their biocompatibility and low toxicity

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(Hasan, 2015).2) Inorganic Nanoparticles: These include both metal and metal oxide nanoparticles. Metal-Based: Silver (Ag), copper (Cu), iron (Fe), gold (Au), and zinc (Zn) NPs are widely used due to high reactivity and strong antimicrobial properties. Silver NPs are particularly effective in water disinfection. Metal Oxide-Based: Iron oxide (Fe_2O_3), magnetite (Fe_3O_4), titanium oxide (TiO_2), zinc oxide (ZnO), and others are used in catalysis and water purification systems.3) Carbon-Based Nanoparticles: Include fullerenes, carbon nanotubes (CNTs), graphene, carbon black, and nanofibers. Fullerenes (e.g., C_{60}) have a cage-like carbon structure. CNTs are rolled graphene sheets, categorized as single-walled or multi-walled, offering mechanical strength and conductivity. Graphene is a 2D sheet of carbon atoms with excellent electronic properties. Carbon Nanofibers and Black provide high surface area and stability, useful in filtration and reinforcement applications (Al Tammar, 2023; Kumari and Sarkar, 2021). Waterborne Pathogens and Associated Risks: Coliform Bacteria-Coliforms are indicator organisms signaling fecal contamination. While not pathogenic themselves, their presence suggests the risk of harmful microbes in drinking water (Martin et al., 2016). Staphylococcus aureus- S. aureus can shift from a commensal to a pathogenic state, causing severe infections. It evades the immune system through mechanisms such as complement inhibition, phagocytosis resistance, and toxin production. Biofilm formation aids its persistence in medical and aquatic environments (Armstrong & Esther, 1976; Ziebuhr, 2000; Goerke, 2004; Malak et al., 2020). Escherichia coli (E. coli) E. coli is a common gut bacterium, with pathogenic variants such as EHEC, EPEC, ETEC, and UPEC capable of causing diarrhea, UTIs, or even meningitis. These variants possess diverse virulence factors and show considerable genomic plasticity (Kaper et al., 2004). Nanoparticles for Waterborne Pathogen Removal: Various nanoparticles are used to eliminate microbial contamination in water:

Silver Nanoparticles (AgNPs)

Known for broad-spectrum antimicrobial activity, especially against Gram-negative bacteria like E. coli. Their effectiveness is size- and dose-dependent. AgNPs are commonly used in portable water filters and coatings due to their stability and efficiency (Ahmed et al., 2012; Olawade et al., 2024).

Copper Nanoparticles (CuNPs)

Highly effective in disrupting bacterial biofilms formed by pathogens like E. coli and P. aeruginosa. Their anti-biofilm activity enhances water purification (Olawale et al., 2024; Sengan et al., 2018).

Silica Nanoparticles (SiNPs)

Function by adsorption of heavy metals, bacteria, and organic molecules. Functionalized SiNPs offer selective binding capabilities, making them suitable for water remediation (Yehia and Mahmoud Said, 2023).

Carbon Nanoparticles (CNPs)

Their antimicrobial activity, combined with high chemical stability and surface area, makes them ideal for water disinfection (Indarto et al., 2020; Liu et al., 2018).

Iron Nanoparticles (FeNPs)

Used for microbial inactivation and heavy metal removal. Surface modification with surfactants or polymers improves their antimicrobial efficacy and dispersibility in water systems (Aragaw et al., 2021).

METHODS AND MATERIALS

Green Synthesis of Iron Nanoparticles Using Green Tea Leaf Extract:

Sample Collection

Green tea leaves (Lipton Green Tea) were sourced from a commercial vendor.

Preparation of Green Tea Extract

7.8 g of green tea leaves were added to 390 ml of distilled water and heated in a water bath at 80°C for 30 minutes. After heating, the solution was filtered, and the extract was stored in a clean, dry beaker (Fig. 1).

Synthesis of Iron Nanoparticles

0.09732 g of ferric chloride (20 mM) was dissolved in 30 ml distilled water. Equal volumes (10 ml each) of ferric chloride solution and green tea extract were mixed in a sterilized flask (Fig. 2). A black color change indicated nanoparticle formation. The solution was centrifuged at 6000 rpm for 10 minutes, the supernatant discarded, and the pellet dried in a Petri plate to obtain iron nanoparticles (KSV, 2017) (Fig. 2).

Characterization of Iron Nanoparticles

UV-Visible spectroscopy (200–800 nm) confirmed nanoparticle formation. Iron nanoparticles dispersed in DMSO showed an absorption peak around 320 nm, indicating surface plasmon resonance.

Collection of Water Samples for TVC Analysis

Sewage water sample were collected from Sewage Treatment Plant (Kasarwadi). Sample were filtered using Whatman filter paper No. 1.

Total Viable Count (TVC)

Untreated and iron nanoparticle-treated water samples (50 ml) were shaken for 1 hour. The samples were diluted 1:7 in saline, and a 10^{-7} dilution was plated on Nutrient Agar and MacConkey agar. Plates were incubated at 37°C for 24 hours. Percentage reduction was calculated using: % Reduction = (Total Count - CF) × 100 / Total Count, where CF is the colony count after treatment.

Synthesis of Iron Nanoparticle-Based Filter

Iron nanoparticles were incorporated with graphite and activated using 25 ml fuming nitric acid and 50 ml sulfuric acid. 5.0 g of graphite was slowly added, followed by 25.0 g potassium nitrate at 5°C with stirring. The mixture was heated to 70°C for 24 hours and exposed to air for 3 days. Floating material was washed, filtered, and the process repeated four times (Lee and Rickard, 2005).

Filtration Testing

A filter was constructed with a 250 g base of pebbles and sand, followed by a cotton layer. Water samples were filtered through the iron-based filter. A 1 ml aliquot of each filtered sample was serially diluted up to 10^{-5} and plated on Nutrient Agar and MacConkey agar. After incubation at 37°C for 24 hours, bacterial colonies were counted to assess filtration efficiency.

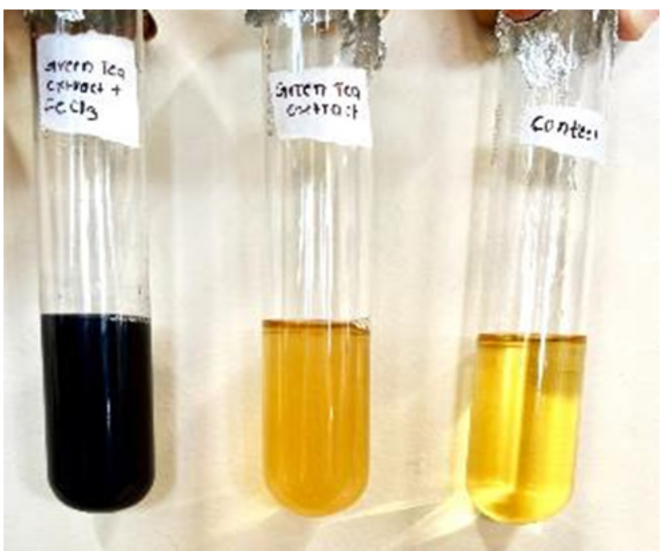
RESULTS AND DISCUSSION

Green Synthesis of Iron Nanoparticles Using Green Tea Leaf Extract:

The green synthesis of iron nanoparticles using green tea leaf extract was successfully achieved, as indicated by a rapid color change from yellowish-brown to black upon mixing the extract with 20 mM ferric chloride solution in a 1:1 ratio. This immediate color shift signified the reduction of ferric ions and the formation of iron nanoparticles. The mixture was then centrifuged at 6000 rpm for 10 minutes to separate the nanoparticles. After discarding the supernatant, the black pellet obtained was dried, resulting in powdered iron nanoparticles. The use of green tea extract provided a natural and eco-friendly reducing and stabilizing agent, effectively promoting nanoparticle synthesis without the need for toxic chemicals. The process was visually monitored at each step, including extract preparation, filtration, and final nanoparticle recovery, confirming the successful formation of iron nanoparticles with consistent yield and appearance.

Characterization of Nanoparticle

The UV-Vis spectra were recorded in the wavelength range of 200 to 800 nm using a UV-Visible spectrophotometer. A small aliquot of the nanoparticle dispersion was placed in a cuvette with a 1 cm path length for the measurement.



Extract +20 mM ferric Chloride (1:1 proportion) | Extract | Control: 20 mM ferric chloride

Fig 1: Synthesis of Iron nanoparticles

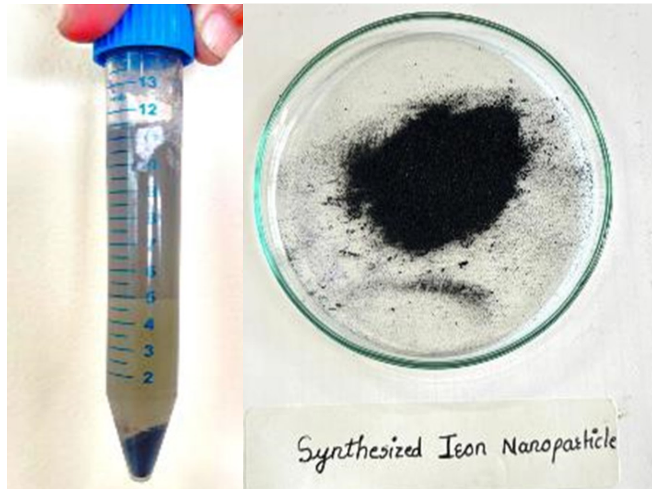


Fig 2: Pellet containing Iron nanoparticles and Iron nanoparticles after drying

Control used: Dimethyl sulfoxide

Iron nanoparticles: Absorption observed between 240-310 nm, with a λmax at 282 nm (Fig. 3).

Collection of water sample for TVC:

Water sample were collected from a sewage source. The sample were subsequently filtered to eliminate impurities.

Treatment of water sample using Iron Nanoparticles:

Sewage Water sample was treated with iron nanoparticles. The 50 ml of treated samples were kept on a rotary shaker for 1 hour.

Total Viable Count (TVC)

The treated and untreated water sample was diluted 1:7 in saline to reduce the concentration of microorganisms. A 10^{-7} dilution of the sample was then spread on Nutrient Agar (NA)

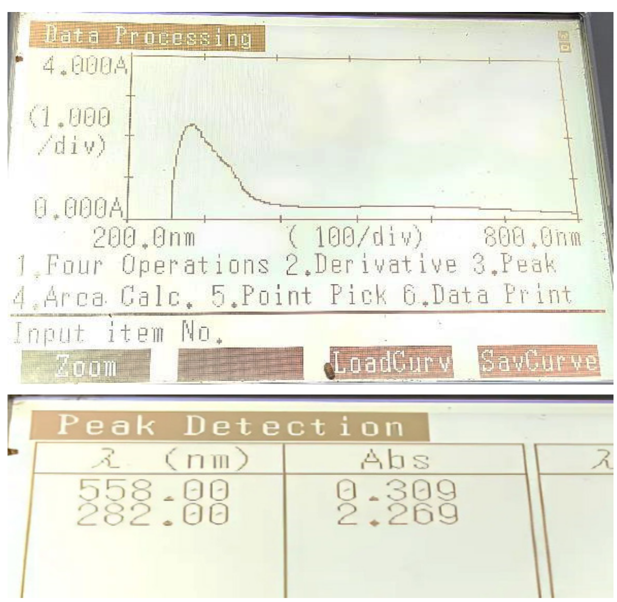


Fig 3: The UV-Vis spectrum of the iron nanoparticles, with λmax at 282 nm

Fig 3: Colonies on NA Media (Untreated Sample)

Fig 4: Colonies on NA Media (treatment using Iron nanoparticles)

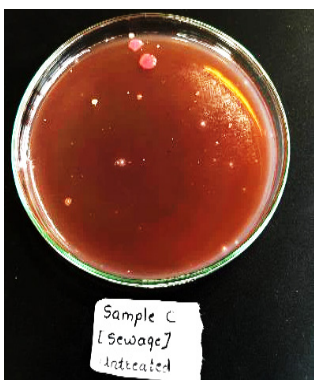


Fig 5: Colonies on MacConkey Media (Untreated Sample)

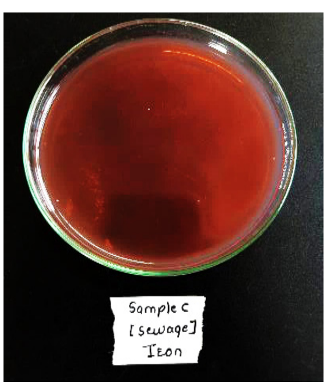


Fig 6: Colonies on MacConkey Media (treatment using Iron nanoparticles)

and MacConkey plates to assess the presence of total viable bacteria and coliform bacteria. The plates were incubated at 37°C for 24 hours to allow the growth of colonies.

Treatment of Sewage water sample using Nanoparticles:

Colony-Forming Units- The percentage reduction in colony formation was calculated using the formula :- (total count-CFU)×100÷Total count, where CFU represented the colony-forming units on the treated samples, and total count referred to the colonies on the untreated samples (Figs 4-7).

CFU percentage for the carbon and iron- treated samples was calculated using NA media9 Tables 1 and 2).

CFU percentage for the carbon and iron- treated samples was calculated using MacConkey agar media (Tables 3 and 4).

Iron based Filter

Graphite (5.0 g) was gradually added to a mixture of 25 ml fuming nitric acid and 50 ml sulfuric acid over 30 minutes, then cooled to 5°C. Potassium nitrate (25.0 g) was slowly introduced while stirring for another 30 minutes. The solution was heated to 70°C and maintained for 24 hours before exposure to air for three days, allowing most graphite to settle. Floating carbon materials were transferred to deionized water, stirred for an

Table 1: Colony Count on Nutrient Agar (CFU/ml)

	Number of Colonies on Media (Nutrient Agar) CFU/ml
	Water sample
Nanoparticles	Sewage
Untreated	35
Iron	04

Table 2: % Reduction rate (Nutrient agar)

	% Reduction on NA media (CFU/ml)
	Water sample
Nanoparticles	Sewage
Iron	88.5

Table 3: Colony Count on MacConkey Agar (CFU/ml)

	Number of Colonies on Media (MacConkey Agar) CFU/ml
	Water sample
Nanoparticles	Sewage
Untreated	20
Iron	04

Table 4: % Reduction rate on MacConkey media (CFU/ml)

	% Reduction on MacConkey media (CFU/ml)
	Water sample
Nanoparticles	Sewage
Iron	80

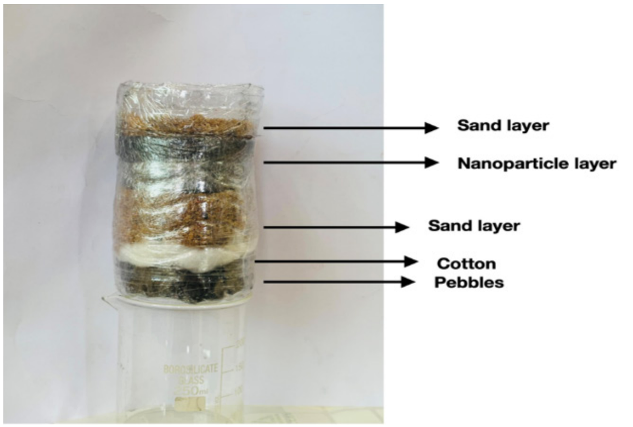


Fig 7: Filter system

Fig 8: Iron Filter system

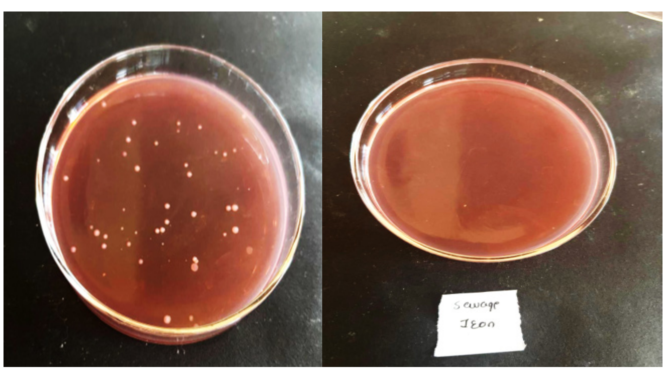


Fig 9: Treatment of sewage water sample using iron-based filter

hour, filtered, and dried (Figs 8-10). This process, including the addition of potassium nitrate and washing, was repeated four times and used for colony count (Fig. 11).

Construction of filter system

The iron-based filter system was developed.

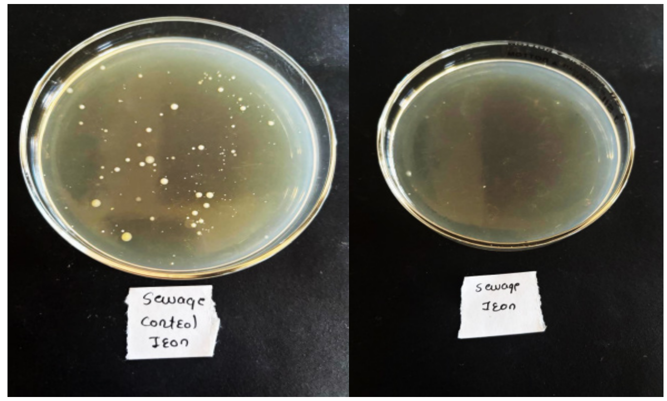


Fig 10: Treatment of sewage water sample using iron-based filter

CONCLUSION AND FUTURE ASPECTS

The green synthesis of iron and carbon nanoparticles offers an eco-friendly, cost-effective, and efficient method for waterborne pathogen removal, avoiding toxic chemicals while ensuring strong antimicrobial activity. Iron nanoparticles (FeNPs) and carbon-based materials like carbon nanotubes and graphene effectively inactivate a broad range of Gram-positive and Gram-negative bacteria through ROS generation, membrane disruption, and enhanced adsorption. Their superparamagnetic and high surface area properties support easy separation, reuse, and long-term stability, making them ideal for integration into filtration systems for municipal, industrial, and household water treatment. Looking ahead, future developments should focus on enhancing nanoparticle selectivity through advanced surface modifications, improving reusability with low-cost regeneration techniques, creating hybrid materials for better adsorption and catalysis, scaling up for large-scale applications, and incorporating smart sensors for real-time monitoring to ensure efficient and sustainable water purification.

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