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RESEARCH ARTICLE

Green Nanoremediation of Phytogenic Copper Oxide Nanoparticles for Concurrent Organic Pollutant Degradation in Industrial Wastewaters and Antibacterial Activity

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ABSTRACT

In the present investigation, culture A. niger was used for the biosynthesis of CuO-NPs using mango (Mangifera indica) leaves aqueous extract. This method of green synthesis is simple, more environment friendly and easy to scaled up. The obtained CuO-NPs were characterized using FT-IR, XRD, TEM, EDX and DLS to evaluate their structure, morphology, elemental composition, and particle size distribution. These findings verify the generation of spherical, crystalline CuO-NPs with an average size of 35.1 nm. The biosynthesized plant-based CuO-NPs showed potential antimicrobial and catalytic, with the higher concentration (till 6.0 mg/mL) having effect better the effect. When used for wastewater treatment, under solar light, the NPs exhibited superior decolorization with a performance of 80.1 $\pm$ 1.5% at 6.0 mg/mL after 250 min, which is better than the 61.3 $\pm$ 1.3% observed in the dark. Moreover, the physicochemical parameters, viz., (TSS), (TDS), (COD) and (BOD) were also decreased significantly by 90.93%, 82.59%, 91.2% and 82.65%, respectively in the best process condition. Also the concentrations of heavy metals (cobalt, Co; lead, Pb; nickel, Ni; cadmium, Cd; and hexavalent chromium, Cr (VI)) were significantly reduced up to 69.9%, 79.7%, 75.0%, 77.0%, and 91.2%, respectively. Antimicrobial analysis demonstrated the CuO-NPs at the minimum inhibitory concentrations (MICs) of 100, 25, 100, 100, 25, and 50 μg/mL against Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae, correspondingly. To sum up, mango leaf mediated synthesis of CuO-NPs acts as potent antibacterial agent as well as a catalyst for the wastewater treatment so as to their application in sustainable treatment of industrial effluent.

Keywords: Antibacterial, Green synthesis, Heavy metals, Leather effluent, Mango leaves

Introduction

Nanotechnology is based on the design and synthesis of nanomaterials, is one of the fastest growing areas in contemporary science. Nanoparticles, with size ranging from 1 to 100 nm, can exhibit novel or improved properties related to those of their bulk materials due to the small size.[1–3] These particles are playing an increasingly prominent role in enabling innovative sustainable solutions for the good of human health and the environment. One of the potential solutions is the green synthesis of nanomaterials using plants, which integrates nanotechnology and plant biotechnology.[4–6] The multifunctionality of

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NPs has resulted in their applications in agriculture, biomedicine, and other fields[7] due to their unique physicochemical properties like large surface area to volume that promote their reactivity.

Biosynthesis is emerging as a green alternative to physical and chemical methods for nanoparticle synthesis.[8,9] Microorganisms such as bacteria, fungi, actinomycetes, and algae have been proven to be capable of synthesizing sustainable nanoparticles (silver, gold, platinum, iron) and metalloids such as titania and silica.[10,11] In view of the increasing need for green synthetic routes, recent reviews emphasize on the potential of biological entities (microbes/plants) for production of nanoparticles using an economical, green, and sustainable approach.[12] Polysaccharide-Based Processes Green synthesis methods, such as those using polysaccharides as stabilizing and reducing agents, differ immensely from traditional chemical methods, which usually contain toxic and hazardous substances.[13] The main aspects in the synthesis of green nanoparticles is to use water based solvents and non-toxic capping agents.[14]

The leaves, fruit, peel and seed of the mango tree (Mangifera indica), a major tropical fruit from the Anacardiaceae family, are known to possess wide array of phytochemicals. Medicinal properties of the above-listed are attributed to the presence of mangiferin, gallic acid, quercetin, ascorbic acid, and a lot of other polyphenols.[15] Mango seed kernels are especially good sources of antioxidants, antimicrobial and anticancer compounds which can find utilization in pharmaceutics.[16–19] As a result of high reducing ability, some part of mango such as leaves and peels have been used successfully to synthesize CuO-NPs.[20,21]

The plant extract polysaccharides and proteins are absorbed on the surface of NPs as capping agents by binding to the surface of NPs through functional groups like OH, C=O, NH2 groups providing steric stabilization and inhibiting the agglomeration. Similar mechanisms of action of mango-derived phytochemicals in synthesis of NPs have been reported by Yadav et al. (2022) and Donga & Chanda (2021).[22,23]

Copper and copper oxide nanoparticles (Cu-NPs/CuO-NPs) have attracted tremendous interest in recent years due to the numerous applications in the field of agriculture, medicine and environmental cleanup. These nanoparticles stimulated plant growth/development and disease resistance, as well as having antimicrobial, antiviral and anticancer effects.[24] These adsorbents have high surface area, which makes them promising for efficient adsorption and application in wastewater treatment. Also, their reusability and cost-effectiveness make them especially favorable for application in developing countries.[25] Factory wastewater, such as tannery wastewater, is loaded with toxic chemicals including dyes, heavy metals and organic compounds, which are destructive to the environment and human health.[26]

Conventional treatment processes are incomplete, indicating the application of nanotechnology to enhance pollutant removal. For instance, CuO-NPs have attained 94.5% efficiency in the elimination of chemical oxygen demand from polluted water.[27] In addition, biosynthesized CuO-NPs have been used in the removal of dyes up to 90% as well as heavy metals like nickel, chromium, and lead from textile wastewater,[28] Significant portion of subjected dyes in textile wastewater also crystallizes CuO-NPs at room temperature. The impact of pH, contact time and nanoparticle dose on the purification of metal ions by them is also discussed.[29] Magnesium oxide nanoparticles (MgO-NPs) derived from fungi have also displayed excellent performance for the recovery of tannery effluents by decolorizing more than 95% of color and almost 97% of chromium ion along with significant improvement in water quality gauges including TSS, TDS, COD, and BOD.[30] Therefore, green synthesized nanoparticles offer a non-hazardous, environment friendly and biocompatible approach for industrial wastewater treatment as well as heavy metals remediation.

In this study, CuO-NPs were green synthesized using mango leaf extract and characterized by using FT-IR, XRD, TEM, EDX, DLS, and SEM. Rutin was investigated for the first time as a potential natural agent for deco lorization of wastewater, physicochemical enhancement, and heavy metal removal in combination with CuO-NPs as nanoadsorbents for tannery effluent treatment. Furthermore, the biongenic CuO-NPs were evaluated for their antibacterial activity on different bacterial strains.

Materials and methods

Preparation of leaf extracts

Healthy, green Mangifera indica leaves were harvested from the garden of the College of Science for Women, without any sign of disease. Subsequently, the leaves were washed under running tap water, shade dried for two weeks and next stored. For the preparation of aqueous extract, 10 g of the dried leaf powder was suspended in 400 mL of sterile distilled water in a 500 mL beaker. The solution was boiled 10 minutes at 100 °C and hanged the color from transparent to brown-yellow. The obtained extract was filtered using whatman no. 1 filter paper following by centrifugation at 1200 rpm for 5 minutes to separate any residual plant material. The purified solution was kept for later use in experiments.[20]

CuO-NP biosynthesis via M. indica leaf extracts

Preparation of CuO-NPs: 2.8 g of copper acetate monohydrate was dissolved in 500 mL of deionized water and stirred magnetically at room temperature for 5 min to synthesize the CuO-NPs. Subsequently, an aqueous M. indica leaves extract was added dropwise to the solution with constant stirring. The solution changed color from blue to green on addition of the copper ions, which is a visible reduction of the process. This reaction resulted in stable, water-soluble, and monodispersed copper oxide nanoparticles[20] within 10 min.

Characterization of bioinsired CuO-NPs

The shape and size of the plant extract-mediated CuO-NPs were examined by using high-resolution microscopy. For TEM studies, the powder of nanoparticles was dispersed in an aqueous solution by ultrasonication and a drop of this solution was placed on a carbon-coated TEM grid. Quantitative size analysis was performed by evaluating more than 100 individual particles across multiple TEM images via ImageJ software (v1.52a, NIH, USA).[31] Complementary morphological examination was conducted through scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX, JEOL JSM-6360LA, Tokyo, Japan).[32] Crystalline properties were determined via X-ray diffraction analysis via a PANanalytical X'Pert Pro MRD diffractometer with Cu Kα radiation operating at 40 kV and 30 mA. Diffraction patterns were recorded across a 2θ range of 10–80°.[33] For functional group analysis, FT-IR spectroscopy (Cary-660 model) was employed in the 4000–400 cm^{-1} range. The samples were prepared by homogenizing approximately 10 mg of CuO-NPs with KBr and compressing them into pellets.[34] Colloidal properties were evaluated via dynamic light scattering (DLS, Nano-ZS, Malvern Instruments, UK). The nanoparticles were suspended in ultrapure water (Milli-Q) to minimize interference during the measurements. The surface charge characteristics were determined via the same instrument's zeta potential measurement capability.[35]

Evaluation of the catalytic performance of CuO-NPs in wastewater treatment

Degradation and decolorization of industrial tanning effluent were used as a parameter to evaluate the photocatalytic efficiency of green CuO-NPs. The experiments were performed in the 1–6 mg/mL concentration range and various exposure times (25–250 min) in dark and under sunlight. All experiments were conducted at 37 °C, pH change was monitored before and after treatment. Aerated aeration was applied with a high-speed blower and medium-bubble diffusion in the experimental equipment. Before catalytic experiments, the desired concentrations of NPs were mixed with wastewater samples and stirred (150 rpm) for 30 min to reach adsorption-desorption equilibrium. Control experiments in dark and under natural light were run parallel and each treatment was carried out in three replications, to ensure the statistical reliability. The decolorization activity was determined by monitoring the absorbance at 550 nm using a spectrophotometer (721-Spectro, M-ETCAL) after subjecting periodic samples (1 mL) to centrifugation (10,000 rpm; 5 min). The degradation rate was determined using the following equation:

$$\text{Decolorization efficiency (\%)} = \left(\text{Initial absorbance} - \text{Time-point absorbance}\right) / \text{Initial absorbance} * 100.$$ [36]

Extensive water quality testing was conducted on samples in the best conditions of treatment. The tannin content, pH, COD, BOD, TDS and TSS were analyzed by the standard procedures.[37] The concentration of heavy metals (Pb, Co, Ni, Cd and Cr) was also determined using an atomic absorption spectrophotometer (PerkinElmer Analyst 800) and compared with the baseline concentrations of untreated effluent.

Antibacterial activity

Antibacterial activity of environmentally benign CuO-NPs was screened on six clinically important bacterial isolates by using standard microbiological methods. The species studied were of both gram-positive (Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC 6538 and Enterococcus faecalis ATCC 29212) and gram-negative (Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027 and Klebsiella pneumoniae ATCC 13884) categories.[38]

Bacterial cultures were incubated at 37 °C for 24 h on Nutrient agar. For determination of the antibiotic resistance profile, few colonies were picked up by sterile swabs and spread over all Mueller-Hinton agar plates (Oxoid) using the entire cotton surface of the swab for evenly distribution. Wells (diameter 0.7 mm) were formed in the agar medium and 100 μL of nanoparticle suspensions at concentrations between 6.25 and 200 μg/mL were added to the wells. DMSO was used as the solvent to suspend the CUO-NPs and served as the negative control, as it has no antimicrobial activity at the concentrations used (negative control) or copper acetate solution (positive control). Following a 24-hour incubation at 37 °C, antibacterial activity was quantified by measuring the inhibition zone diameter (mm) surrounding each well. The minimum inhibitory concentration (MIC) was determined as the lowest nanoparticle concentration producing visible growth inhibition.[39] All the assays were conducted in triplicate, and the results are expressed as the means ± standard deviations.

Statistical analysis

Statistical analysis was performed via GraphPad Prism (version 8). One-way and two-way ANOVA were used to evaluate the significance of the differences between groups. Differences were considered statistically significant at p values < 0.05.

Results and discussion

CuO Nanoparticle synthesis via M. indica leaf extract

The current study employed an eco-friendly approach for synthesizing CuO-NPs from aqueous extracts of *M. indica* leaves. The reaction was monitored visually, whereby the initial blue copper acetate solution turned green after approximately 10 min of magnetic stirring. This change in color was an initial indication of the successful generation of nanoparticle) and it reflects that CuO NPS formed could be water-soluble and monodispersed.[20] M. indica leaves have an interesting chemical pattern made up of several bioactive constituents including Polyphenolic compounds (flavonoids, alkaloids), organic acids, polysaccharides, proteins along with vital micronutrients.[40] These in-situ reducing agents have bifunctional capabilities during the nanoparticle generation process, whereby they assist in the reduction of Cu^{2+} ions to metallic copper (Cu°) and then serve as capping agents for stabilizing the resulting nanoparticles.[41]

Characterization of bioisbired CuO-NPs

The X-ray diffraction study was carried out for the analysis of crystalline structure of phytosynthesized CuO nanoparticles. The XRD pattern exhibited distinct diffraction peaks at 2θ angles of 33.75° (110), 36.63° (–111), 38.23° (111), 49.49° (–202), 53.89° (020), 58.58° (202), 61.22° (–113), 68.39° (220), and 75.93° (–222), as illustrated in Fig. 1. These diffraction peaks are consistent with the face-centered cubic crystal structure of CuO reported in reference pattern (JCPDS card no. 80–1268).[42] The specific peaks detected at 35 to 39° of 2θ show a positive sign of CuO nanoparticles, which is well agreement with the previously reported data.[43] The XRD pattern showed several significant characteristics of the prepared

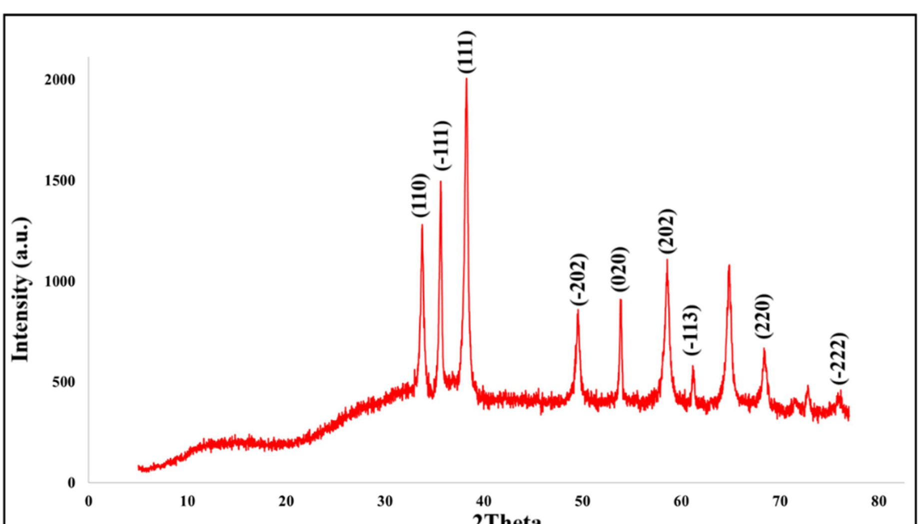

Fig. 1. X-ray diffraction patterns of biofabricated CuO-NPs.

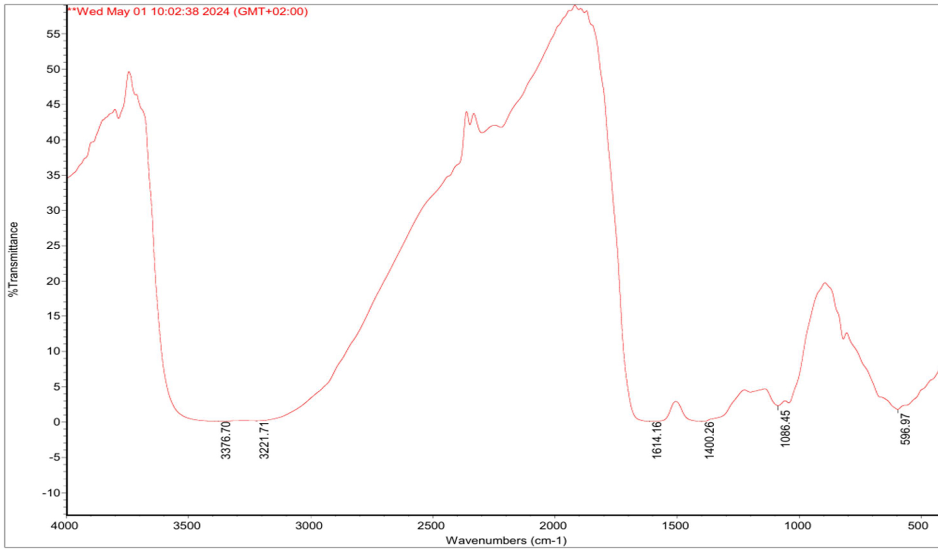

Fig. 2. FTIR analysis of biofabricated CuO-NPs.

nanoparticles. No extra peaks indicated that the biosynthesized CuO nanoparticles were highly pure as revealed by EDX. The sharp, well-defined peaks indicate excellent crystallinity, with crystallite sizes less than 100 nm.[44] Several analyses with previous studies have demonstrated that crystallite size varies depending on the plant extract used for synthesis, as Azadirachta indica-derived CuO nanoparticles are approximately 7 nm in size.[45] In addition, Prunus africana-mediated synthesis is nearly 43 nm in length. Camellia sinensis-based preparations are approximately 38 nm long.[46] The XRD data are consistent with that found in various accounts of green-mediated CuO NP synthesis, indicating the successful growth of crystalline nanoparticles by this plant mediated green method. The narrow peak widths and high intensity of diffraction patterns indicate superior crystallinity and phase purity of the prepared material. The results are very important, since they indicate that plant metabolites can act as efficient agents to produce highly crystalline metal oxide NPs without using hard chemical precursors.

Fourier transform infrared spectroscopy was used to ascertain the biomolecular components responsible for green synthesis and stabilization of copper oxide nanoparticles. The FT-IR spectrum of the synthesized CuO NPs showed absorption bands at 3376, 3221, 1614, 1400, 1086 and (596 andε406 cm^{-1}) Fig. 2. The wide bands at 3376 and 3221 cm^{-1} could be assigned to O–H stretching vibrations of the hydroxyl groups, but may also involve N–H stretching modes from primary amines.[47] A predominant peak at 1614 cm^{-1} presents amide functionalities (primary and secondary) from the proteins, as well as carbonyl stretching vibrations related to polysaccharides 41. Other spectral features include a separate band at ∼1086 cm^{-1} assigned to C-O-C stretching for the carbohydrate moieties.[48] Characteris tic metal » oxygen vibration s in range of 400–700 cm^{-1} correlated well with Cu»O bond formation confirming the successful attachment of Cu.[46,49] The components of proteins, polysaccharides and amide containing compounds in the plant extract were found through spectroscopic study to have played different functions such as aiding the reduction of copper acetate precursors, mediation for the nanoscale structure formation and surface stability by binding interactions. These results provide evidence that the phytochemical components not only play a role in the intracellular metal ion bioconversion but also help stabilize the resultant nanoparticles for longer durations through surface passivation. The found functional groups are in good agreement with the known reducing and stabilizing features of plant extracts produced metabolites regarding nanoparticle formation.

The structure and composition of green-prepared CuO nanoparticles play a critical role in their biological behaviour. Sophisticated characterization methods such as TEM, SEM, EDX, and DLS were used to investigate these important parameters. Spherical nanoparticles were observed as a well-dispersed form with little aggregation under a microscopic examination Fig. 3A-C. Size distribution measurement indicated the diameters are widely distributed from 24 to 51.2 nm, and the average size was 35.1 nm. Comparative studies have shown size-dependent on

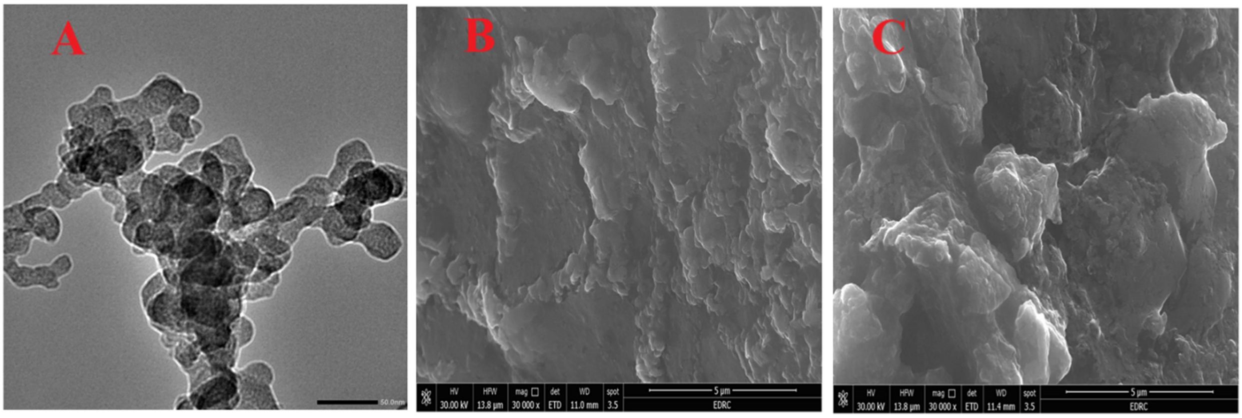

Fig. 3. Morphological characteristics of biofabricated CuO-NPs (A: TEM & B, C: SEM).

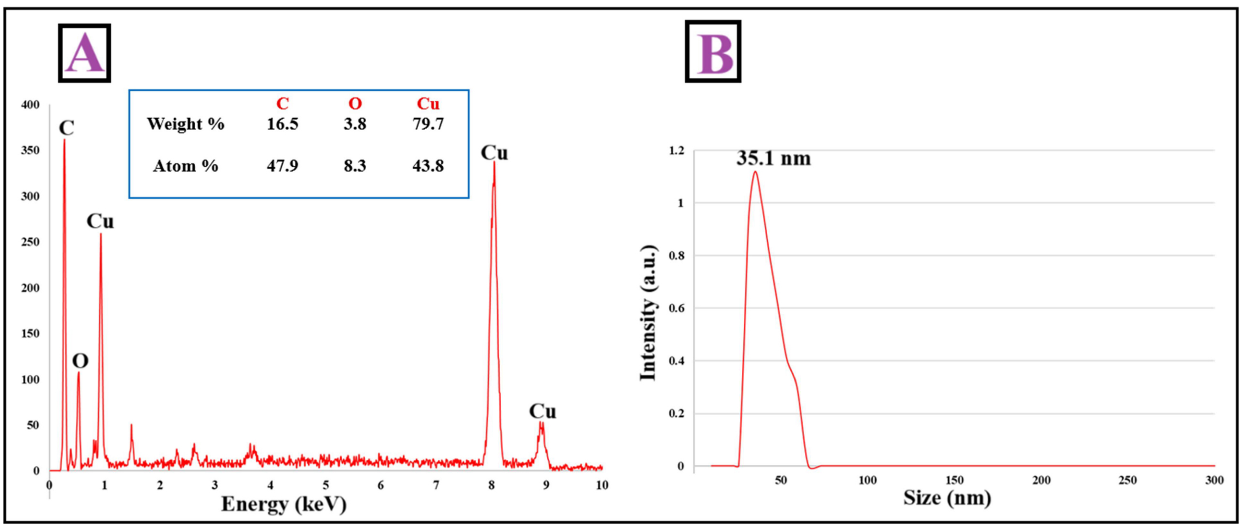

Fig. 4. The main components and mean sizes of the biofabricated CuO-NPs (A: EDX and B: DLS).

the plant extracts where the nanoparticles were synthesized in which the particle sizes are ranging from around 16 to 31 nm for Annona muricata L. 46 and from pumpkin seed extract via using a standard addition method results a particle size of approximately 20 nm.[50] The bioreactivity of nano-materials highly relies on their physical properties. For instance, cytotoxic studies showed that 24-nm CuO NPs are more toxic to A549 adenocarcinoma cells compared to 4-nm NP irrespective of faster release of copper ion by smaller particles.[51] In addition, nonspherical morphologies prepared using Aloe vera extract are more effective in antibacterial activity against E. coli and S. aureus compared to spherical nanoparticles.[52] These experiments indicate that surface architecture and energy distribution are important for biological interactions.[53] Spherical shape and smooth surficial topography of the biosynthesized nanoparticles have been confirmed by SEM analysis Fig. 4 A, B that are in convergence with their previous reports when mint leaves and orangepeel extract has been used.[29] Elemental composition was confirmed by EDX spectroscopy, which revealed Cu- and O-specific signals at 1.0/8.0/9.0 keV and 0.5 keV, respectively Fig. 4A. Quantitative analysis gave values (%wt) of 79.7Cu, 3.8O and (atomic %) Cu 43.8O8.3%S 47.9 and Cl1/40!6%n (n1/42). The detected carbon signal (16.5 wt%) presumably comes from the organic capping agents plant extract.[29] These results are consistent with the findings of previous studies with *Oryza sativa*[45] and *Azadirachta indica*.[45] extracts while possessing superior purity in the sense that it has less additional elemental impurities than systems at 1.3Tc that are not extracted.[46] The DLS measurement results showed an average hydrodynamic diameter of 35.1 nm Fig. 4B, which was consistently

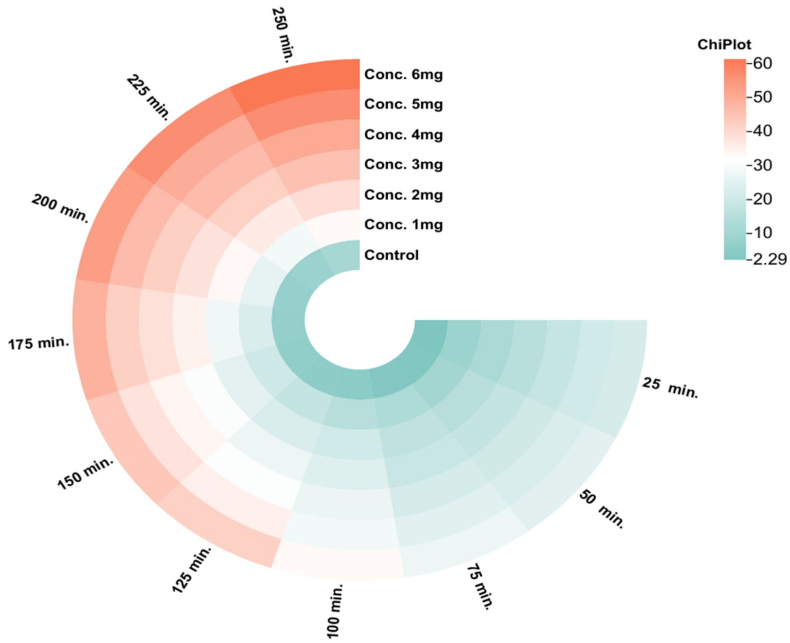

Fig. 5. Heatmap showing decolorization percentages of tanning wastewater after treatment with different concentrations (1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mg/mL) of CuO-NPs at various intervals (25 min to 250 min) under dark incubation conditions, where high decolorization is shown in orange and less decolorization is shown in green. The plot was generated via ChiPlot (Chiplot.online).

higher than those from TEM and XRD measurements. This disparity is due to basic measurement discrepancies. TEM analyses dry particles, and DLS measures particles in solution as hydrated nanoparticle. There are many factors that can affect the results of DLS, such as solution homogeneity, organic shell thickness, and measurement conditions. These differences in measurement values were observed between such studies, both of which used the same plant species as a source of nanoparticles: Enicostemma axillare[54] found that this alternative method leads to NPs 6.4 nm (TEM) and 470 (DLS) nanometres in size. Analogous findings have been documented on the nanoparticles obtained from Camellia sinensis (6–61 nm) and Prunus afana (8–68 nm) through various characterization methods.[46] These results highlight the necessity of performing multiple techniques in complementary for nanoparticles characterization.

Tannery wastewater treatment

The worldwide water shortage has made the exploration for new technologies to treat industrial effluents more important, especially in arid regions of the world. Leather tanneries Lin et al. Incorporating harmful elements such as Pb and Cd, those treated improperly present a serious threat on the ecological systems[55] and human public health. In this sense, green nanotechnology has been developed as an attractive alternative for the synthesis of environmentally friendly, efficient and stable nanomaterials with excellent surface properties to remove pollutants.[56] The antimicrobial properties of these nanomaterials provide the added benefit of both wastewater decontamination and inactivation of microbes. Five time points (25, 50, 150 and 250 minutes) and four concentrations of nanoparticls (1.0, 2.5, 4 and 6mg/ml) were used in a comprehensive experimental design. These findings revealed that decolorization efficiency of the nanoparticles was directly related to concentration and time, due to more active surface sites in higher nanoparticle loadings.[57,58] Decolorization observed in control experiments was negligible (2.3–11.3%), whereas 9.1–32.8% removal was achieved after treatment with 1.0 mg/mL CuO-NPs. Higher activity was noted at higher concentrations, and 4.0 mg mL −1 treatment resulted in 18.5–50.3% decolorization Fig. 5. Other similar studies have also reported significant degradation of the dye using green synthesized nanomaterials where 16.1–97.5% decolorization was obtained by MgO nanoparticles (0.25–1.0 μg/mL, 30–180 min). There is even 79.2% for the removal ratio adsorption of γ-Fe_2O_3 nanoparticles at 30 = 1.0 mg/mL after 144 min. Best dark conditions showed 61.3% decolorization (6.0 mg/mL, 250 min). The degradation efficiency was significantly promoted by sunlight irradiation, and the removal efficiencies were 60.6–80.1% at a concentration of

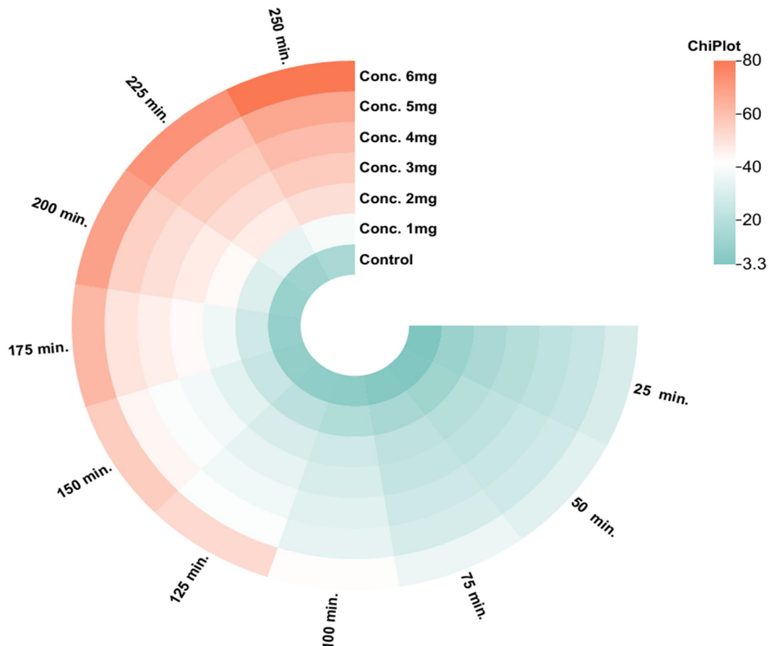

Fig. 6. Heatmap showing decolorization percentages of tanning wastewater after treatment with different concentrations (1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mg/mL) of CuO-NPs at various intervals (25 min to 250 min) under sunlight incubation conditions.

Table 1. Physicochemical parameters of wastewater after treatment with CuO-NPs under optimum conditions (6.0 mg/mL for 250 min under sunlight irradiation conditions).

Physicochemical Parameter	BeforeTreatment (mg/L)	After Treatment with 6.0 (mg/mL) under Sunlight Condition	Removal Percentages(%)
Tannin	1752	163.7	90.65
pH	8.75 ± 0.2	6.8 ± 0.25	–
COD	952.3 ± 3.5	86.3 ± 2.4	90.93
BOD	2356.5 ± 4.55	410.2 ± 1.75	82.59
TSS	6542.5 ± 3.12	573.4 ± 2.14	91.23
TDS	2156.2 ± 1.42	374.1 ± 1.54	82.65
CO	2.47 ± 0.8	0.742 ± 0.75	69.95
Pb	1.953 ± 0.54	0.395 ± 0.42	79.77
Ni	2.45 ± 0.75	0.612 ± 0.87	75.02
Cd	0.856 ± 0.15	0.196 ± 0.24	77.1
Cr	842.5 ± 1.7	82.2 ± 1.16	90.24

4.0–6.0 mg/mL Fig. 6. This photocatalyticactivity isthe result ofthe generation electron-hole pairs upon absorption of a photon, and the subsequent formationof reactive oxygen speciesand oxidative decompositionof the pollutant intoharmless byproduct.[59]

Wastewater parameter assay

The treatment efficiency was also assessed based on extensive physicochemical evaluation at optimum conditions (6.0 mg/mL, 250 min of sunlight exposure). The tannin, COD, BOD, TSS and TDS were eliminated by the biogenic CuO-NPs with 90.65%, 90.93%, 82.59%,91.20% and 82.65%, respectively Table 1. After treatment, the pH of alkaline wastewater (pH = 8.75) was neutralized to pH = 6.80. These values are comparable to those reported in the literature, where maghemite NPs offered 88.8% COD degradation[60] and different pool of CuO-NP concentrations (0.1–30 mg/L) showed 89.5–91.3 % COD removal.[61,62] The treatment efficacy is found to be highly insensitive to the tested nanoparticle concentration ranges, indicating the ability of achieving optimal performance for a wide range of operational conditions. These results place plant-mediated CuO nanoparticles as promising and economically sustainable alternative for full treatment of tannery wastewater, combining physical-chemical-biological purification modes in a single-eco-friendly route. Comparison with previous studies can be found in Table 2.

Evaluation of nanocatalyst stability by reusability test

The assessment of nanocatalyst stability has been regarded as a key factor for the practical feasibility on an industrial scale, in particular in wastewater treatment.[63] In the present study, the reusability analysis of biosynthesized CuO NPs was carried out in a methodical way under optimized operational conditions (6 mg mL−1 concentration of catalyst, 25 min time of exposure, and use of sunlight irradiation). The performance evaluation demonstrated stepwise decrease in the catalytic activity over several treatment cycles. The initial decolorization yield at 89.7 ± 0.2% during the first application cycle was preserved. However, on subsequent uses there was a 18.06% decrease in the treatment efficacy after six cycles of sequential treatments and the final performance parameters stabilize at 71.6 ± 1.7% Fig. 7. The present findings suggest that the CuO-NPs have good reusability (only a ∼18% decrease in catalytic activity after being used six times), which could contribute to better economic and environmental performance of wastewater treatment methods.

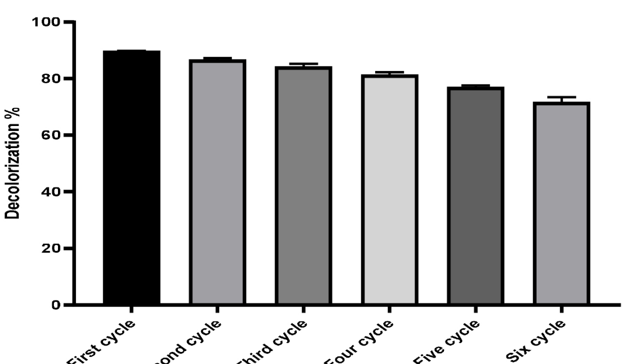

Fig. 7. Reusability test for investigating the ability of green-synthesized CuO-NPs to decolonize wastewater for several cycles.

Remediation of heavy metal contamination

Due to their long biological half-lives, bioaccumulation and acute toxicities in living systems, the heavy metal poisoning is one of the serious environmental concerns. Such non-degradable pollutants accumulate in terrestrial and aquatic environment, and they represent a significantly threat to human health, flora, and fauna via various exposure pathways.[64] Although rock weathering is one of the intrinsic geological mechanisms that drive metal mobilization, it has been reported that anthropogenic factors, especially industrial sources, vehicle emissions and mining activities are the fundamental factors responsible for heavy metal pollution in the environment.[64] The pollution load of the leather tanning industry is particularly worrisome, releasing effluents with high-level toxic metal contents. Analytical characterization of untreated tannery effluent in this study revealed substantial quantities of chromium (842.5 ± 1.7 mg/L), along with measurable levels of cobalt (2.47 ± 0.8 mg/L), lead (1.953 ± 0.54 mg/L), nickel (2.45 ± 0.75 mg/L), and cadmium (0.856 ± 0.15 mg/L) Table 1. The greenish-blue colour of this waste water is a visual indication that the complete potential of chromium has been used up during leather manufacture.[55] Newest achievements in green nanotechnology provide hopeful answers for heavy metals removal. Engineered nanomaterials exhibit excellent metal scavenging properties as a result of its large surface area and adjustable surface functionality.[30] In the present study, under the optimal conditions of 6.0 mg/mL for CuO-NPs, removal rates were: chromium(VI), $\alpha = 91.2\%$; lead: $\alpha = 79.7\%$; cadmium: $\alpha = 77.0$; nickel: $\alpha = 75.0$; and cobalt: $\alpha = 69.9\%$. Such separation can be ascribed to the numerous surface adsorption sites on and the high metal coordination capacity of the nanoparticles. This is supported by comparative studies where the CuO-NPs removed 75–92% of mercury and chromium from aqueous systems.[65] Phytomediated synthesis methods for NPs that included the use of orange peel and mint leaf extracts were perhaps as good with respect to Pb, Ni, Cd detoxification.[30] It is important to mention that the composition of the nanomaterial plays a crucial role in the effectiveness of these treatments. Catharanthus roseus extract mediated AgNPs resulted in 47.8% removal of chromium but their cadmium adsorbing ability was restricted to 5.7%66. The same plant extract developed CuO-NPs with lower metal affinity (2.1–2.9% of removal)[66] that emphasizes the criticality of material choice for adsorbates to be targeted.

Antibacterial potential of CuO-NPs

The increasing prevalence of antibiotic-resistant microbial strains poses a significant threat to global health, contributing to increasing morbidity and mortality rates. This challenge has spurred the exploration of novel antimicrobial agents, with nanotechnology offering promising solutions owing to the unique physicochemical properties of nanoparticles.[67] In the present study, the efficacy of the biosynthesized CuO-NPs was evaluated against clinically relevant gram-positive (*B. subtilis* ATCC6633, *S.*

aureus ATCC6538, and *E. faecalis* ATCC29212) and gram-negative (*E. coli* ATCC8739, *P. aeruginosa* ATCC9027, and *K.* pneumoniae ATCC13884) pathogens via an agar well diffusion assay. The data indicates a concentration-dependent antimicrobial activity and the highest inhibition zones were recorded at 200 μg/mL, which were: B. subtilis (21.7 $\pm$ 0.64 mm), E. faecalis (24.6 $\pm$ 1.0 mm), S. aureus (18.1 $\pm$ 0.52 mm), Pseudomonas aeruginosa (15.2 $\pm$ 0.35mm) E.coli (23.4$\pm$ 0.45 mm and Klebsie lla pneumoniae (20.5 $\pm$ 0.76 mm) Fig. 8. These values were higher than those of the bulk precursor compound ($p < 0.05$), confirming previous results on the increase of antimicrobial activity over nanomaterials.[52] Numerous studies further validated these results, with CuO-NPs synthesized from *Camellia sinensis* and *Prunus africana* extracts exhibiting inhibition zones of 26–30 mm against *E. coli, K. pneumoniae*, and *S. aureus* at 250 μg/mL.[46] Similarly, rod-shaped CuO-NPs derived from Momordica charantia demonstrated broad-spectrum activity (25–32 mm inhibition zones) against multiple bacterial strains, including Streptococcus spp. and *Proteus vulgaris*.[68] Although copper ions (Cu^{2+}) are recognized by the US Environmental Protection Agency as effective antimicrobial agents,[69] the present findings confirm that compared with their bulk counterparts, nanoformulated Cu/CuO-NPs exhibit superior activity,[70] likely due to their increased surface area and enhanced cellular penetration.

The present investigation revealed a clear dose-response relationship with the antimicrobial activity of the green-synthesized CuO nanoparticles. Quantitative analysis revealed progressively diminishing inhibition zones with decreasing nanoparticle concentrations, as evidenced by reduced zones of 16.5 $\pm$ 0.32 mm (*B. subtilis*), 18.6 $\pm$ 0.64 mm (*E. faecalis*), 12.0 $\pm$ 0.3 mm (*S. aureus*), 11.4 $\pm$ 0.25 mm (*P. aeruginosa*), 16.1 $\pm$ 0.64 mm (*E. coli*), and 14.8 $\pm$ 0.26 mm (*K. pneumoniae*) at 100 μg/mL Fig. 8. Determination of MICs established crucial thresholds for clinical applicability, with plant-derived CuO-NPs exhibiting MIC values of 100 μg/mL for the gram-positive organisms *B. subtilis, S. aureus* and *P. aeruginosa* and demonstrating enhanced potency against gram-negative strains (25 μg/mL for *E. faecalis* and *E. coli* and 50 μg/mL for *K. pneumoniae)*. The differences in susceptibility between classes of bacteria were observed and could be understood if the cells had different basic shapes. The difference in resistance of gram-positivenegative compared to gram-negativenegative species is consistent also with the finding that these have thin cell walls (with respect to peptidoglycan, a thick layer),[71] indicating that structural barriers strongly govern nanoparticle uptake efficiency.

The greater sensitivity of *E. coli* and *E. faecalis* to CuO-NPs, as indicated by their lower MIC values, may be attributed to differences in cell wall structure and oxidative stress tolerance. In *E. coli*, outer membrane porins facilitate the uptake of nanoparticles and release Cu^{2+} ions, increasing their intracellular accumulation, whereas in *E. faecalis*, the relatively porous peptidoglycan layer allows easier penetration and interaction with the cytoplasmic membrane. CuO-NPs exert their antimicrobial activity primarily through the generation of reactive oxygen species (ROS), which induce oxidative damage to essential biomolecules, and direct disruption of bacterial membrane integrity, ultimately leading to cell lysis and death.[72]

These findings align with established structure-activity relationships for nanomaterials, where reduced particle dimensions correlate with enhanced antimicrobial effects.[51,52] Multiple synergistic mechanisms contribute to the biocidal activity of CuO-NPs, as intracellular dissolution releases Cu^{2+} ions that chelate sulfhydryl groups in essential amino acids and disrupt protein function.[32] Compared with endogenous metal cofactors, metalloprotein activity is impaired.[73] The catalytic generation of reactive oxygen species induces lipid peroxidation, nucleic acid strand breaks and enzyme inactivation.[74] Table 2 shows a comparison of the performance of TiO2, ZnO or CuO NPs.

Table 2. Comparison between our findings and those of previous studies (TiO_2, ZnO, CuO NPs).

Nanomaterial	Reusability cycles (Final efficiency %)	Removal efficiency (%)	Target pollutant/application	Method used	References
CuO-NPs (this study)	Cycles (∼71.6%) 6	90.93, (color) 80.1 (CDO)	Tannery wastewater (Color + CDO	Green synthesis using *M. indica* extract	Current study
CuO-NPs	Not reported	92–75	Heavy metals (Pb, Ni, Cd)	*Catharanthus roseus* extract	75
TiO_2-NPs	Cycles (∼65%) 5	90–85	Dye degradation (Methylene blue)	Sol-gel	76
ZnO-NPs	Cycles (∼70%)4	88–82	Textile wastewater	Green synthesis (*Cassia javanica*)	5
CuO-NPs	Not reported	85–76	Dye degradation (Congo red)	*Prunus Africana* extract	46

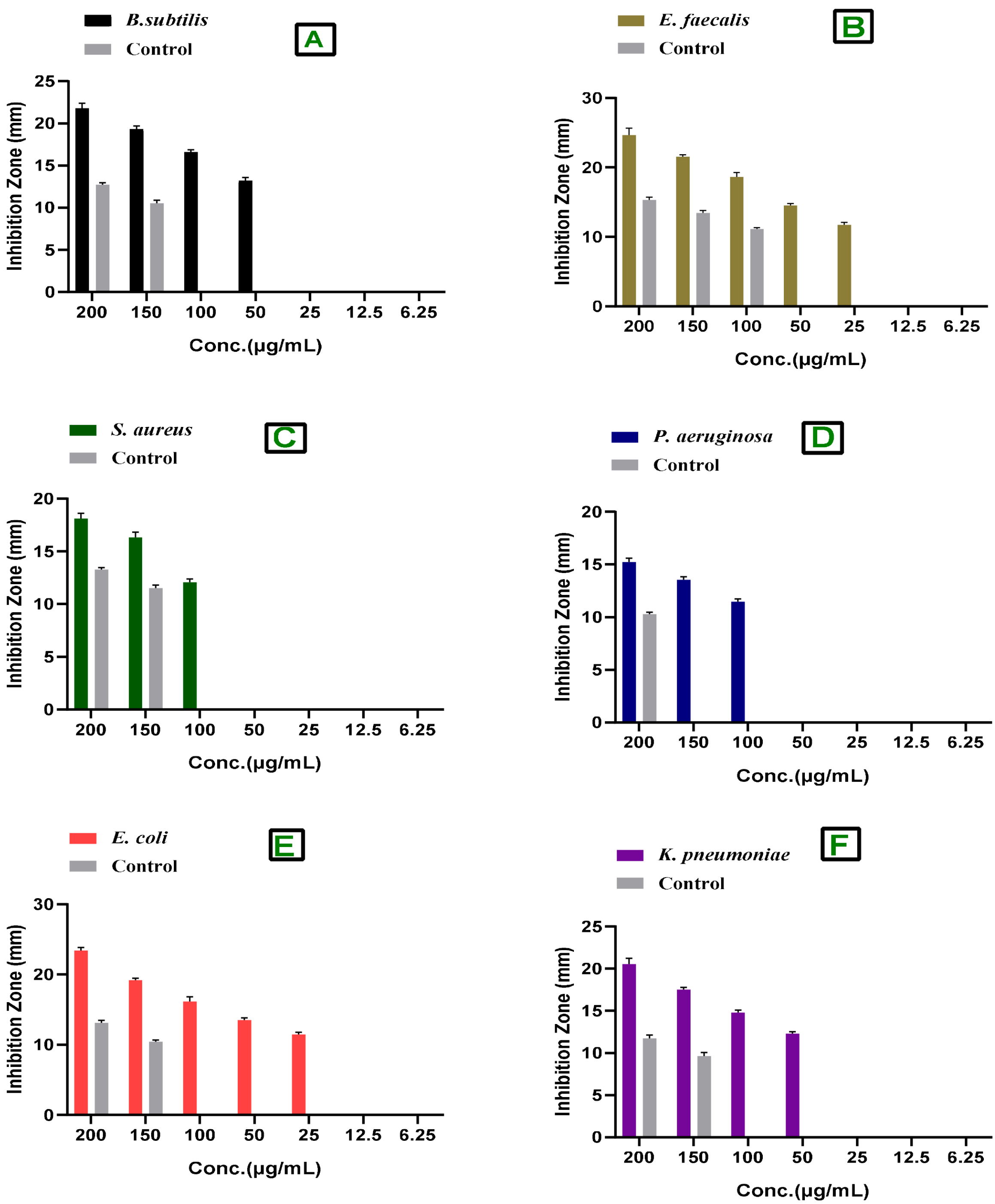

Fig. 8. Antibacterial activity of bioinspired CuO-NPs toward *B. subtilis* (A), *E. faecalis* (B), *S. aureus* (C), *P. aeruginosa* (D), *E. coli* (E), and *K. pneumoniae* (F). The mean values are significantly different (p value < 0.05).

Conclusion

Our study successfully demonstrated an eco-friendly biosynthesis approach for CuO-NPs using *M. indica* leaf extract as a reducing and stabilizing agent. DLS characterization revealed spherical nanoparticles with an average hydrodynamic diameter of 35.1 nm. Evaluation of the catalytic performance of CuO NP nanoparticles revealed a decolorization efficiency of 61.3% under dark conditions. This catalytic efficiency substantially increased to 80.1% when the samples were exposed to solar irradiation. Moreover, CuO-NPs exhibited exceptional heavy metal removal capabilities, with reduction efficiencies of 69.9% for cobalt, 79.7% for lead, 75.0% for nickel, 77.0% for cadmium, and 91.2% for hexavalent chromium in wastewater samples. Moreover, the effectiveness of tannery wastewater was significantly remarkable in CuO-NP treatment as reduction rates for tannin contents, COD, BOD, TS and TDS were 90.65%, 90.93%, 82.59%, 91.2% and 82.65%; respectively. Simultaneously, the biologically synthesized CuO NPs exhibited remarkable antimicrobial potential against broad spectrum of pathogenic bacteria like B. subtilis, E. faecalis, S. aureus, P. aeruginosa, E. coli and K. pneumoniae with MIC values ranging between 25–100 μg/mL. Based on the results of this study, we highly recommend that these biosynthetic NPs are used in industrial wastewater treatment as well as for microbial management applications.

Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images that are not ours have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Authors' contribution statement

A. H. T. designed the study W. H. A. performed the experiments performed simulations and expressed and purified all proteins S. A. S. A. and Z. A. A. analyzed the data wrote the paper with input from all authors.

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Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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المعالجة النانوية المستدامة لجسيمات أكسيد النحاس النانوية النباتية للتحلل المتزامن للملوثات في النفايات الصناعية والفعالية المضادة للبكتيريا

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الخلاصة

في هذه الدراسة، تم تخليق جسيمات أكسيد النحاس النانوية (CuO-NPs) بيولوجيًا باستخدام مستخلص مائي من أوراق المانجو، مما يوفر نهجًا فعالًا من حيث التكلفة وصديقًا للبيئة وقابلًا للتطوير. وُصفت جسيمات أكسيد الinoaطاس النانوية المُخلَّقة باستخدام تقنيات تحليلية متنوعة، بما في ذلك مطيافية تحويل فوربيه بالأشعة تحت الحمراء (FT-IR)، وحيود الأشعة السينية (XRD)، والمجهر الإلكتروني النافذ (TEM)، ومطيافية الأشعة السينية المشتتة للطاقة (EDX)، والتشتت الضوئي الديناميكي (DLS). أكدت النتائج تكوين جسيمات أكسيد النحاس النانوية الكروية البلورية بمتوسط حجم 35.1 نانومتر. أظهرت جسيمات أكسيد النحاس النانوية المشتقة من النبات تأثيرات مضادة للميكروبات ونشاطًا تحفيزيًا ملحوظًا يعتمد على التركيز. عند تطبيقها على معالجة مياه الصرف الصحي الناتجة عن الدباغة، أظهرت الجسيمات النانوية تحسنًا ملحوظًا في إزالة اللون تحت أشعة الشمس، محققةً كفاءةً بلغت 80.1 ± 1.5% عند تركيز 6.0 ملغم/مل بعد 250 دقيقة، مقارنةً بـ 61.3 ± 1.3% في ظروف مظلمة. بالإضافة إلى ذلك، انخفاض المعايير الفيزيائية والكيميائية الرئيسية - مثل إجمالي المواد الصلبة العالقة (TSS)، وإجمالي المواد الصلبة الذائبة (TDS)، والطلب الكيميائي للأكسجين (COD)، والطلب البيولوجي للأكسجين (BOD) - بشكل ملحوظ بنسبة 90.93%، و82.59%، و91.2%، و82.65%، على التوالي، في ظل الظروف المث من الجدير بالذكر أن المعالصكقة أعالص الكوة تركيزات المعادن الثقيلة، بما في ذلك الكوبالت (Co)، والرصاص (Pb)، والنيكل (Ni)، والكادميوم (Cd)، والكروم ((Cr (VI)، بمعدلات انخفاض بلغت 69.9%، و79.7%، و75.0%، و77.0%، و91.2% على التوالي. وكشف التقييم المضاد للميكروبات أن جسيمات أكسيد النحاس النانوية (CuO-NPs) ثبطت نمو البكتيريا المسببة للأمراض مثل العصوية الرقيقة (B. subtilis)، والإشريكية البرازية (E. faecalis)، والمكورات العنقودية الذهبية (S. aureus)، والزائفة الزنجارية (P. aeruginosa)، والإشريكية القولونية (E. coli)، والكليبسيلا الرئوية (K. pneumoniae) عند تركيزات تثبيطية دنيا بشكل عام، يُقدم التخليق الصديق للبيئة لجسيمات أكسيد النحاس النانوية باستخدام مستخلص أوراق المانجو مادة نانوية واعدة مزدوجة الوظيفة، تتميز بخصائص مضادة للبكتيريا قوية وكفاءة تحفيزية عالية، مما يجعلها حلاً مستدامًا لمعالجة مياه الصرف الصحي.

الكلمات المفتاحية: مضاد للبكتيريا، التخليق الأخضر، المعادن الثقيلة، نفايات الجلود، أوراق المانجو.