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Heavy Metal Detection and Antibacterial Activity of Phytosynthesized Copperoxide (CuO) Nanoparticles Using Leaf Extract of *Colocasia esculenta*

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ABSTRACT

Phytosynthesis of metal nanoparticles offers a facile and stable approach for their production and applications. In this study, CuO nanoparticles (CuO-NPs) were effectively synthesized using *Colocasia esculenta* plant leaf extract as a capping and bio-reducing agent. The spectroscopic analysis, XRD, confirmed the crystalline hexagonal primitive structure of the CuO-NPs with a crystal size of 42.14 nm, whereas the UV-VIS spectroscopy reflected the absorption peak at 260 nm. The CuO-NPs displayed significant potential for detecting and removing Pb^{2+} heavy metal from water with a detection limit of 29 μM. CuO-NPs exhibited excellent sensitivity and selectivity for Pb^{2+} in the presence of several interfering metals. The adsorption isotherm displayed the Langmuir working model for Pb^{2+} adsorption. Prominent antibacterial activity was also indicated by the phytosynthesized CuO-NPs against Gram-positive and Gram-negative bacteria, suggesting their prospective role for antimicrobial applications. Thus, the study highlights an easy, cost-effective, non-toxic way of synthesis of CuO-NPs utilizing the leaf waste of *Colocasia esculenta*, offering an insight into their probable applications for heavy metal detection and antimicrobial activity.

Key words: CuO-NPs, *Colocasia esculenta*, phytosynthesis, heavy metal, detection, antibacterial

INTRODUCTION

Agricultural processes produce a significant amount of agricultural waste. The issue of disposing of agro-waste could be resolved by using agricultural crop wastes in the extraction of value-added products. Nanotechnology has the highest level of efficacy in managing the issue of agro-waste. Agricultural waste may now be permanently transformed into recyclable raw materials for various purposes, as well as an essential component for synthesizing nanomaterials, because of developments in nanotechnology (Gupta and Chandra 2020, Maji and Pal 2024a). *Colocasia esculenta* leaves are considered a by-product after fruit cultivation and discarded as environmental waste. The leave constituents are rich in phenolic acids (derivatives of coumaric, gallic, and caffeic acid) and flavonoids (primarily luteolin, apigenin, and chrysoeriol glucosides) which function as a reducing and stabilising agent for the formation of metal nanoparticles. The most widely used metal nanoparticle is copper oxide nanoparticle (CuO-NP) due to their wide range of applications in optical, electrical, catalytic, antibacterial, and antifungal fields. It has long been known that copper possesses antibacterial properties. Its effectiveness and low toxicity are crucial in the biomedical industry (Barman et al. 2021). Copper oxide nanoparticles have been synthesized using a variety of physicochemical techniques. However, these methods have many disadvantages, including high energy demand, great expense, and the harmful release of toxicant compounds. As a result, more economical and environmentally friendly sustainable nanoparticles with high levels of uniform size, shape, purity, texture, and phase selectivity are required (Gupta and Chandra 2020). Additionally, nanotechnology may provide a reliable and affordable means of improving the consistency and quality of water. It is a potentially helpful method of treating wastewater in drinking water production and irrigation to eliminate contaminants, including pathogenic microbes. The primary goal of the current work is to illustrate a novel method of synthesizing CuO-NPs from *Colocasia esculenta* leaves, an agricultural waste. Furthermore, the biogenic CuO nanoparticles are methodically studied for their uses in the detection of heavy metals and in removing pathogenic microbes.

MATERIAL AND METHODS

Materials

Colocasia esculenta leaves were collected from plants at JIS University, Kolkata. The chemicals, including $CuSO_4.5H_2O$, $NiCl_2.6H_2O$, $HgCl_2$, and $Pb(CH_3COO)_2$, were purchased from Merck. The calculated amounts of these metal salts were mixed with deionized water to prepare a stock solution.

Biosynthesis of copper oxide nanoparticles

A 5 mM aqueous solution of $CuSO_4.5H_2O$ was prepared to synthesize Copper nanoparticles. 25 mL of *Colocasia* leaf extract was added to 80 mL of 5 mM $CuSO_4.5H_2O$ solution dropwise with constant stirring for the bioreduction process at room temperature. The mixture was incubated at 30°C for 24 hrs. The change in colour of the solution from light greenish yellow to deep brownish after 24 hrs primarily indicates the formation of the nanoparticles. The solution was evaporated to dryness using a rotatory vacuum evaporator at 60°C for 30 mins or until dry.

Characterization of nanoparticles

The CuO-NPs were characterized by X-ray diffraction (XRD) (Advanced Bruker D8, CuKá radiation, λ = 1.5408 Å) to study the phase and crystalline structure of the materials. The XRD diffraction pattern was recorded at room temperature within the angular range 2θ = 10°-80°. The optical properties were examined by UV-Vis spectroscopic technique in the 200–600 nm range using CARY-5000 UV-Visible spectrophotometer.

Agar well diffusion and microdilution method

To study the antibacterial effect of CuO-NPs against *E. coli, B. subtilis, S. typhi*, and *S. aureus*, soft nutrient agar was made to inoculate with 200 µl log phase bacteria. The antibacterial effect was visualized after 24 hrs at 37°C by spotting different CuO-NPs concentrations (100-12.5 mg/ml) over soft agar. After incubation, the zone of inhibition was observed and measured (mm). The microdilution method was executed on 96well polystyrene plates to determine the MIC and MBC of CuO-NPs against bacterial strains. To initiate the experiment, overnight bacterial cultures (OD600nm of 0.1-0.2) and the CuO-NPs were tested to determine the minimum NP concentration required for antibacterial activity. Each microtiter plate well was filled with nutrient broth, respective bacteria solution, and CuO-NPs. The plates were incubated at 37°C for 24 hrs, and bacterial growth was determined. MIC was the lowest concentration of NPs with no visible bacterial growth, and MBC was the higher dilution preceding MIC value.

RESULTS AND DISCUSSION

Structural and optical analysis

The XRD pattern of biogenic CuO-NPs (Fig. 1) reveals diffraction peaks at 2θ values 34.1°, 39°, 46°, and 50° which corresponds to the plane of reflections [002], [111], [112], [202], respectively. The data obtained agree with JCPDS file 80-0076, confirming the crystalline CuO with a hexagonal primitive structure (Tan et al. 2020). The absence of any undesired peak confirms the complete conversion of the metal salt precursor into oxide. The average crystal size is 42.14 nm, according to Debye-Scherrer's equation. A similar crystal size was reported with *Ocimum sanctum* synthesized CuO-NPs by Maji and Pal (2024b). Figure 2 shows the UV absorption spectra of synthesized CuO-NPs at 260 nm, indicating nanoparticle formation confirmation.

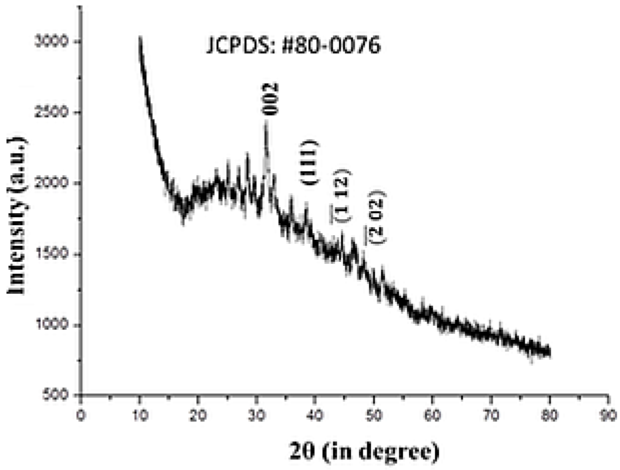


Figure 1. XRD spectra of phytosynthesized CuO-NPs

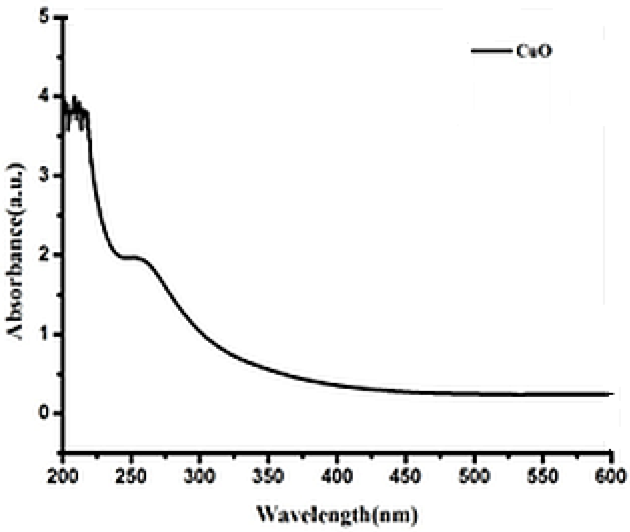


Figure 2. UV-Vis absorption spectra of phytosynthesized CuO-NPs

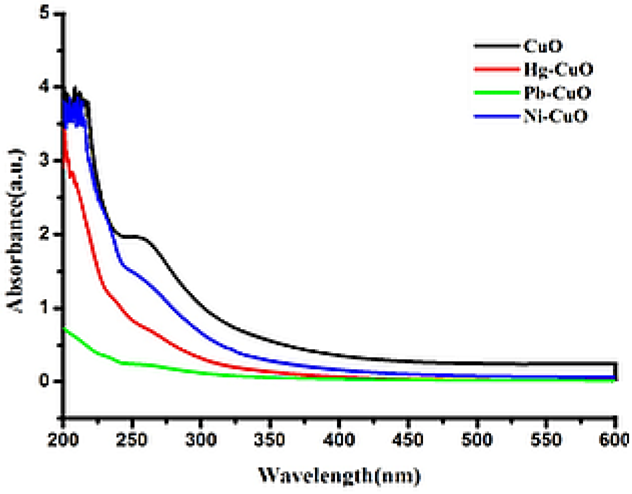


Figure 3. Selectivity of CuO-NPs toward Pb^{2+} in presence of other metal cations

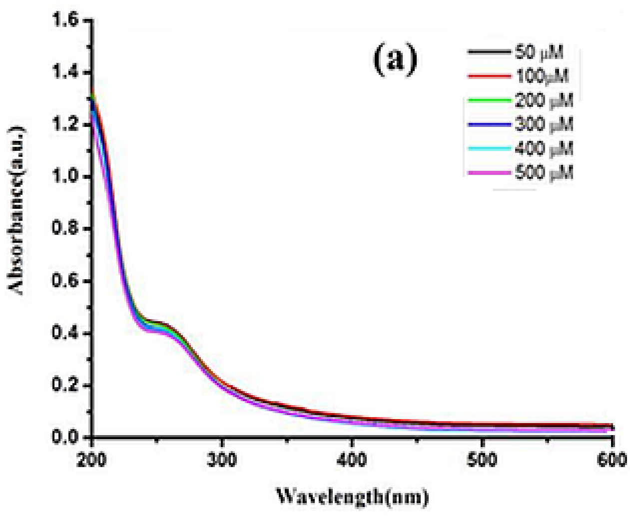
Selectivity and sensing analysis towards the heavy metals

Colorimetry and absorption spectroscopy were performed to investigate the sensing capabilities of the newly synthesized CuO-NPs. 1.0 mL of CuO-NPs solution was added to 1.0 mL of different metals (1 mM) at room temperature for this sensing application, and the changes were recorded (Fig. 3). It was observed that CuO-NPs could detect only the Pb^{2+} metal ion solution within 15 min, visible as a precipitate. No changes were seen in the Hg^{2+} and Ni^{2+} ions.

To investigate the Limit of Detection (LOD), UV-Vis absorption spectra of CuO-NPs solutions in contact with different Pb^{2+} concentrations (50-500 µM) exhibited LOD at 29 µM (Figs. 4a, b). A linear correlation value was observed between CuO-NPs and Pb^{2+} with R^2 at 0.998 (Fig. 4b). The formula LOD = 3 SD/slope was used to calculate the detection limit.

Langmuir and Freundlich model analysis

In order to determine the adsorbent's adsorption capability, the isotherm data analysis is crucial, guided by the Langmuir and Freundlich isotherm



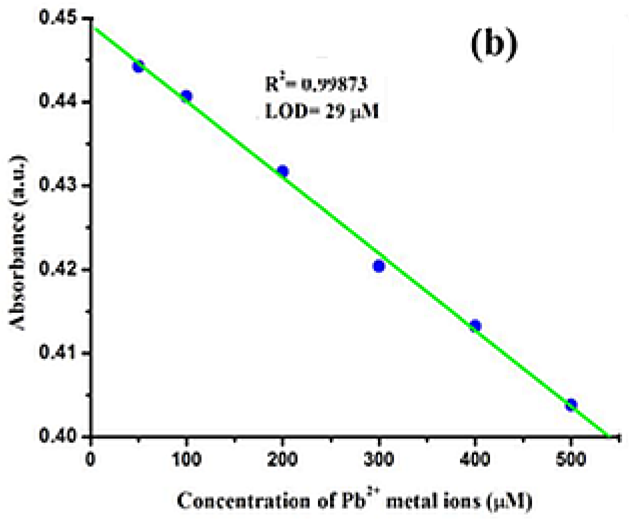


Figure 4. (a) Variation of the absorbance of CuO-NPs solution as a function of Pb^{2+} ion concentration, (b) UV–visible spectra of CuO-NPs showing LOD of Pb^{2+} ion with varying concentration (50-500 µM) in aqueous solution

model. Langmuir model proposes monolayer adsorption on a homogeneous surface (Equation 1), whereas Freundlich indicates adsorbant behavior on the heterogeneous surface (Equation 2).

$$\frac{C_e}{q_e} = \frac{1}{K_L q_{max}} + \frac{C_e}{q_{max}} \text{.................. Eqn (1)}$$

$$\ln(q_e) = \ln(K_f) + n\ln(C_e) \text{............ Eqn (2)}$$

Here, an adsorbent dose of 0.3 g/L at pH 7.0 was mixed with appropriate adsorbates, and the results were analyzed. The adsorption isotherm is based on the concept that all adsorption sites are equal and surrounding sites do not influence a particle's binding ability. Monolayer adsorption is displayed in the Langmuir model, where solutes can occupy similar sites in infinite numbers. The Freundlich isotherm model displays multilayer adsorption on an uneven adsorbent surface (Mandal et al. 2021). The estimated values of the parameters for the Freundlich and Langmuir models are displayed in Table 1. The Langmuir model fits the experimental adsorption equilibrium data better than the Freundlich isotherm model and is the most acceptable isotherm to represent the equilibrium data (Fig. 5). Similar works were reported by Maji and Pal(2024b) where Freundlich isotherm exhibited best-fit data for the adsorption of Pb^{2+} metal ion using CuO-NPs (Maji and Pal 2024b).

Table 1. Isotherm parameters and correlation coefficient for Pb^{2+} Heavy metals using CuO NPs

Langmuir model	
q_{max} (mg g^{-1})	495.04
K_L (L mg-1)	0.0164
R^2	0.9999
Freundlich model	
K_f(mg g^{-1})	9.4737
n_f	0.8633
R^2	0.9998

Antibacterial activity

The inhibition zone of *B. subtilis* on soft agar against CuO-NPs was highest compared to other bacteria tested (Table 2). Moreover, the higher the concentration of synthesized NPs (100 mg/ml), the greater the inhibition zone, probably due to the availability of more copper oxide NPs to generate reactive oxygen radicals to inhibit bacteria (Behzadi et al. 2017, Akintelu et al. 2020, Maji et al. 2023). Our results demonstrate CuO-NPs are more effective against Gram-positive bacteria than Gram-negative (Fig. 6). Similar results were shown in previous studies where better performance of *B. subtilis* was observed than *E. coli* and *S. aureus* (Murthy et al.

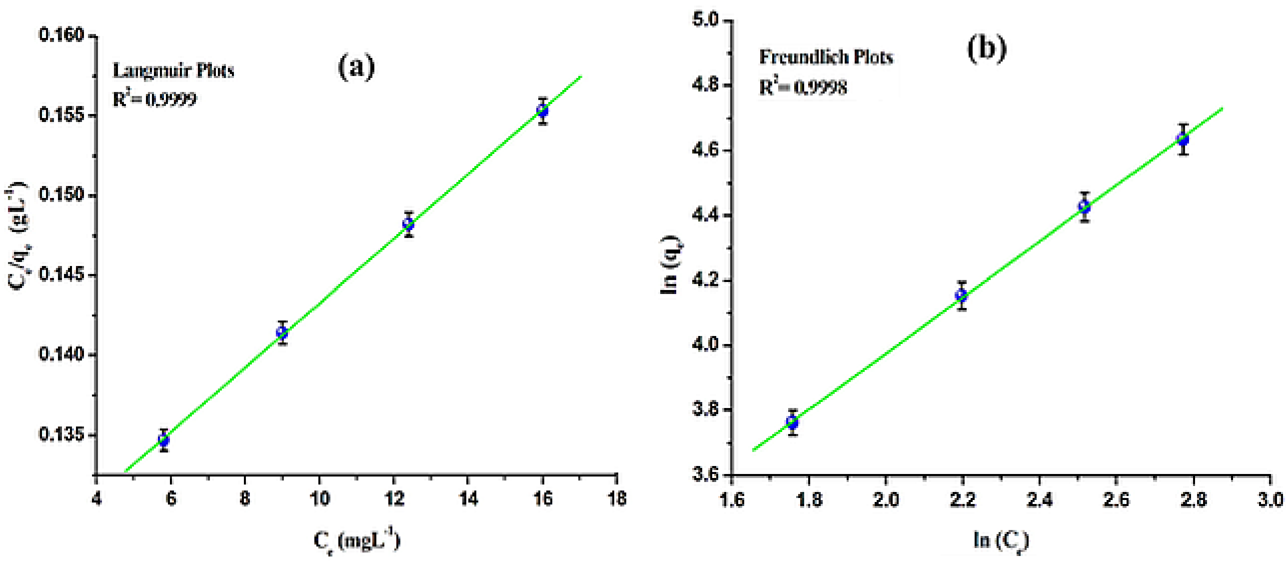


Figure 5. (a) Langmuir plot (b) Freundlich plot displaying the adsorption of Pb^{2+}metal ion using CuO-NPs

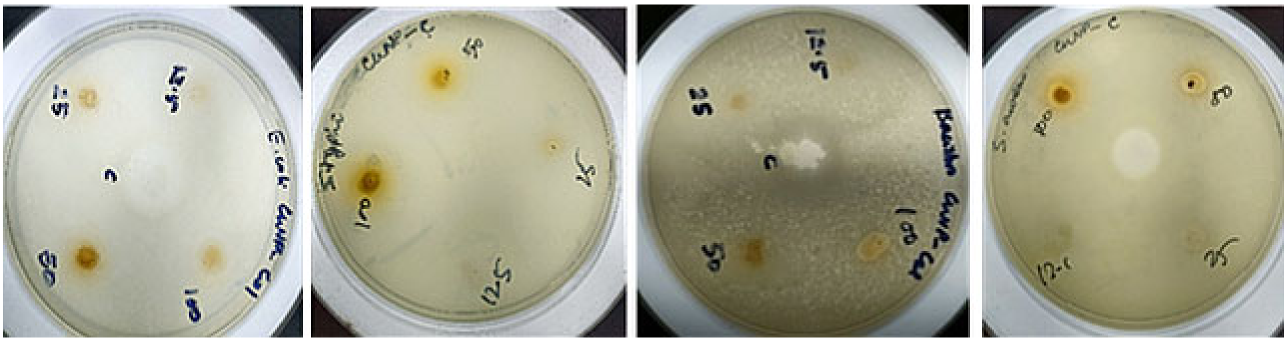


Figure 6. Inhibition zone exhibited by CuO-NPs against a) *E. coli*, b) *S. typhi*, c) *B. subtilis,* d) *S. aureus*

Table 2. Zone of inhibition (mm) against different bacterial species

	CuO-NPs (mg/ml)			
	100	**50**	**25**	**12.5**
B. subtilis	9.05	8.1	7.0	6.0
S. typhi	8.0	7.0	5.0	4.0
S. aureus	8.0	7.1	6.0	4.0
E. coli	8.05	6.0	5.1	4.0

Figure 7. 96 well plate exhibiting the MIC and MBC of different bacterial species

2020, Maji et al. 2023, Kolahalam et al. 2022). The MIC value was 200 and 400µg/ml (MBC) for *B. subtilis*proving greater susceptibility to CuO-NPs than other bacterial strains tested (Fig. 7). Thus, the antimicrobial activity exhibited by CuO-NPs could be used further as a suitable remedy against different bacterial strains.

CONCLUSIONS

The facile synthesis of CuO-NPs using *Coclocasia* leaf waste was described in this study. It was employed to detect Pb^{2+} ions in wastewater. Antibacterial activity displayed prominent inhibition against several tested bacterial strains of medical importance. Adsorption processes indicate the Langmuir model as the best fit for heavy metal ion adsorption following the multilayer process. The synthesized CuO-NPs can be applied for bioremediation and biomedical purposes based on their visible range of utilities.

Authors' contributions: All authors contributed equally.

Conflict of interest: The authors declare no conflict of interest while doing this study.

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