Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Eco-Friendly Synthesis of Copper Oxide Nanoparticles Using Syzygium Cumini Leaf Extract and Their Inclusion in Nanocomposites with Melamine: Morphological and Antimicrobial Studies

Yaqoob Khan
Department of Chemistry, Hazara University Mansehra.
yaqoobkhan22113@gmail.com
Bakht Zada
Department of Chemistry, Hazara University Mansehra
Bakht7430@gmail.com
Arshad Ali Khan
Prospective PhD Candidate | Civil Engineer, Swat, Khyber Pakhtunkhwa, Pakistan
arshadkhaan7c@gmail.com https://orcid.org/0009-0009-4296-4784
Sara Murad
Department Chemistry, Abdul Wali Khan University Mardan
Khanzzz72666@gmail.com

ABSTRACT

The considerable environmental hazards resulted from the conventional chemical approaches have triggered the attention of the research community of nanotechnology to investigate the preparation of NPs by using inexpensive greener method of nanomaterials synthesis. In the present study, cupric oxide (CuO) nanoparticles were synthesized by green route employing an aqueous leaf extract of Syzygium cumini plant, which is already known to be rich in natural reducing and stabilizing phytoconstituents (flavonoids, polyphenols, tannins, etc). Melamine modified on the prepared CuO and CuO–melamine nanocomposites were also combined with melamine ($C_3H_6N_6$) to improve the structural stability and antibacterial activity. The prepared nps and its composites were characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and energy dispersive X-ray Spectroscopy (EDX). The SEM and TEM results showed that the particles were nearly spherical to slightly irregular with the average size of 20–200 nm, and the purity of the element (Cu and O) and the successful introduction of CuO and melamine were demonstrated by the EDX. The antibacterial properties of them and 6 pathogenic bacteria (Staphylococcus aureus, escherichia coli. The

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

nanocomposites exhibited significantly enhanced antibacterial activity as compared to CuO nanoparticles and control antibiotics at maximum inhibition zones of 34 mm. These data suggest that the plant-mediated CuO NPs and their melamine composites can be addressed for effective and ecofriendly antimicrobial utilization in biomedicine and environmental applications.

Introduction

Nanotechnology is an ever-evolving field that deals with the manipulation and control of matter in the dimension of approximately 1-100 nm. In this region, extremely high physical, chemical, thermal, electrical, and optical properties that are quite different from those of bulk materials can be obtained. These special traits have made nanotechnology ahead and cutting edge of science in electronics, energy, biotechnology, pharmaceutical, and environmental industries (Tovar-Lopez, 2023). Among the various nanomaterials, nanoparticles (NPs) have gained enormous attention due to their exceptional reactivity, high surface area and tunability of properties. In materials science, nanoparticles lead to remarkably enhanced strength, toughness, and conductivity for most of the commonly used materials, while in biomedicine, they have spawned such advanced types of drug delivery/therapy, imaging agents, and antimicrobial agents. The increasing interest in nanoparticles has provoked a very large research activity, on the synthesis, structural features and the applications (Altammar, 2023).

Traditional methods for the preparation of nanoparticles, such as the physical or chemical methods of sol-gel, thermal decomposition, and laser ablation, require high energy consumption, severe reaction environment, and toxic reagents [3]. This is because of excessive cost and energy extraction requirement and enormous environmental and health hazards in terms of harmful byproduct generation. As an attempt to circumvent these drawbacks, "green synthesis" methods for NP has attracted much attention, with an increasing number of reports showing that NP can be synthesized from the natural system (e.g., plant extracts, bacteria, fungi), which can act as the reducing and/or stabilizing agent (Oulahal, 2022).

Green synthesis TP is an environment-friendly TP and an impulsive TPs synthesis approach consistent with green chemistry principles. It also removes poisonous substances, a high-press or high-temperature environment and etc., with a

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

less damage done no the global environment. Plant mediated process is one of the biological methods due to its very easy, fast, scale up and the abundance in phytochemicals of plants. Phytochemicals such as flavonoids, alkaloids, phenolics, and tannins serve as capping/reducing agents to facilitate the synthesis of the nanoparticles (Xu, 2020).

Copper oxide (CuO) nanoparticles (NPs) are one of the most useful and desirable group of metal oxide NPs. CuO NPs are highly used in catalysis, energy storage devices (lithium ion batteries), gas sensors, solar cells, and biomedicine. Furthermore, their excellent antimicrobial, antifungal and antiviral properties also render them interesting for health care and environmental applications. In addition, CuO nano materials has the benefits of low cost, easy to access and excellent physicochemical properties (low bandgap, high surface area to volume ratio, etc.) (Chen, 2020).

In the presence of increasing environmental concerns and sustainable characteristic of nanomaterials, the current study has focused on the green synthesis of CuO NPs by applying leaf extract of Syzygium cumini (medicinal plant, having antioxidant activity). Its leaves are high in phytochemicals that act as reducing and capping agents, thus no chemical reagent is needed. Moreover, the synthesized CuO nanoparticles are mixed with nitrogen-based material, melamine ($C_3H_6N_6$), to fabricate nanocomposite for its structural and antibacterial applications (Díez-Pascual, 2020).

Objectives of the Study

Major research objectives are:

- Synthesis of Copper Oxide Nanoparticles (CuO NPs) using plant extract of Syzygium cumini According to the plant extract mediated green synthesis method.
- In another application, CuO NPs are employed for the fabrication of melamine composite nanomaterials.
- To investigate the morphological and the structure of the synthesized nanoparticles nanocomposite by means of SEM, TEM and EDX.
- The aim of this work was to study the antimicrobial effect of CuO NPs and

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

CuO NPs melamine-based nanocomposites on selected bacterial strains.

The present study is intended to enrich to the green nanotechnology under new experimental conditions with an efficient, environmentally benign route to the preparation and use of CuO nanoparticles.

Materials and Methods

Chemicals and Materials

Materials All the chemicals and reagents employed in the current study were of analytical grade and used without any further purification. Copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) was obtained from Sigma-Aldrich (99.9%). The reagent copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), 99.9% was the main chemical for the nanoparticle preparation. This salt served as a precursor of copper oxide nanoparticle (CuO NPs). In the preparation of the nanocomposites, we used melamine ($C_3H_6N_6$) as the caping and integrating agent, because of its nitrogen polyheterocycles and coordination behavior. All working solutions were prepared with DI water throughout the entire experiment to exclude potential contaminations (Zhang, 2022).

The leaves of Syzygium cumini (Jamman leaves) are regularly known as the plant material in green synthesis. The leaves were collected from Baffa town in the vicinity of Hazara University, Mansehra, Khyber Pakhtunkhwa, Pakistan. The reason behind selecting this plant is its proven record of phytochemicals, tannins, flavonoids, and polyphenols to be the most potent natural reducing and capping agents (Reddy et al. The collected leaves were washed first with tap water and then with distilled deionized water to remove impurities on the surface and air-dried at room temperature for use (O'Brien, 2020).

The glassware used in all experiments was thoroughly cleaned with distilled deionized water to be sterile and un-contaminated for synthesis and characterization procedures.

Preparation of Plant Extract

Leaves of Syzygium cumini were collected from Baffa. Town in conception located (approximately) at a space of 10 km from Hazara University, Mansehra (Khyber Pakhtunkhwa), Pakistan. The freshly harvested leaves were taken and were selected

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

being freshly plucked and in healthy condition in order to take maximum phytochemical activity. In order to remove dust, dirt and other surface pollutants leaf samples were first washed in tap water, and then washed twice with distilled and deionized water. Leaves were washed by tap water and air-dried at room temperature in the dark to avoid decomposition by direct light or sunlight for heat- or light-labile phytochemicals (Battle, 2020).

Once dried, the leaves were collected for extraction, 20 g of the leaves were then weighed using an analytical balance and were meticulously diced to smaller pieces to increase the surface area of extraction. The pieces were introduced in a 1000 mL erlermeyer flask, and 250 mL of deionized water was placed. This mixture was stirred and heated using a hot plate at 80 °C for 60 min to promote the extraction of the phytochemicals into the aqueous phase (Kumar, 2023).

The solution was filtered to eliminate gross plant material from the solution on Whatman filter paper after heating and after being cooled. To get a concentration extract, the filtered solution was centrifuged at 4500 rpm for 60 min to eliminate the remaining heavy biomass and suspended particles. The clear leaf extract obtained was filtered and transferred into a new conical flask with aluminum foil to prevent photo oxidization and microbial growth. The extract was preserved at room temperature for usage in subsequent nanoparticle green synthesis (Chand, 2020).

The reduction of copper oxide was carried out by using the aqueous extract of Syzygium cumini which acted as both reducing as well as a stabilizing agent due to its high content of phenolics, flavonoids, tannins, and other bioactive compounds.

Table 2.1: Preparation of Leaf Extract

Syzygium cumini leaves	Deionized Water	Temperature (°C)	Stirring	Centrifuge (4500 rpm)
20 g	250 ml	80 °C	60 Minutes	60 Minutes

Synthesis of CuO Nanoparticles

Upon using aqueous extract of Syzygium cumini leaves in conjunction with copper(II) sulfate pentahydrate [$CuSO_4 \cdot 5H_2O$] as the metal source, the green plant-mediated method has been adapted for the production of copper oxide nanoparticles (CuO NPs). To investigate the influence of the precursor concentration on the NPs

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

size and shape, three molar concentrations of CuSO4 solutions (2, 4, and 6 mM) were washed up (Sarkar, 2020).

In order to prepare these solutions, 0.318 g, 0.638 g and 0.957 g of $CuSO_4 \cdot 5H_2O$ were dissolved individually in 50 mL of deionized water (2 mM, 4 mM and 6 mM, respectively). All solutions were magnetically stirred at mild heat (80 °C) to favor solubilization for 60 min (total dissolution of the compound) (Alshehri, 2020).

When the salt solutions had achieved the desired temperatures, 5 mL of Syzygium cumini leaf extract was added drop wise to each solution with intense stirring. Afterwards the reaction mixtures were stirred for a further 40 min at 80°C. The change in colour of the solution was visually observed from blue to yellowish-dark brown along with the reaction and redox reaction of Cu^{2+} ions with the formation of copper oxide nanoparticles (Mohamed, 2020).

After completion of the reactions, the suspensions were cooled to room temperature. The NPs prepared as-synthesised were centrifuged at the speed of 4500 rpm for 60 min to remove the solid CuO NPs that settled at the bottom of supernatant. The resulting pellet was thoroughly washed by deionised water to remove unreacted plant metabolites and salts. The clean nanoparticles were further oven-dried at 120 °C for 3 h to take them as powders for characterizations (Shah, 2024).

The green synthesis approach uses the phytochemicals of the Syzygium cumini leaf extract where it is utilized as reducing and capping agents with the virtue of lacking toxic chemicals and harsh reaction environments.

Table 2.2: $CuSO_4$ Salt Solution Parameters

Sample No	Amount of Salt Used (g)	Volume of Deionized Water (ml)
2.0 mM	0.318	50
4.0 mM	0.638	50
6.0 mM	0.957	50

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

Table 2.3: CuO NPs Synthesis Steps

Sample No	Salt Concentration (mM)	Salt Mass (g)	Leaf Extract (ml)	Temp (°C)	Stirring Time (min)	Centrifuge Time (min)
1	2.0	0.318	5	80	40	60
2	4.0	0.638	5	80	40	60
3	6.0	0.957	5	80	40	60

Synthesis of CuO–Melamine Nanocomposite

In order to improve the functionality and structure stability of the already prepared copper oxide nanoparticles (CuO NPs), the NPs were embedded in the doped nanocomposite-matrix of melamine ($C_3H_6N_6$). Nitrogen-rich melamine was selected as a good electron-donating material because of nitrogen containing amount of melamine and it can be effectively coordinated to CuO NPs (Kim, 2024).

The nanocomposites were prepared by combined 1.0 g of dried CuO nanoparticles with various quantity of melamine as follows:

- 0.252 g (2 mM)
- 0.504 g (4 mM)
- 0.756 g (6 mM)

The weighed melamine was also included in 50 mL of the stabilizing medium used earlier, Syzygium cumini leaf extract, whilst preparing each composite formulation. The melamine extract obtained above was impregnated with CuO nanoparticles. The mixtures were then stirred vigorously and heated on the hot plate at 80 °C for 60 min to ensure the homogenize of melamine with CuO NPs (Zhang, 2025).

The brown suspensions were centrifuged at 4500 rpm and 60 min in order to precipitate the unreacted materials and the suspended impurities after the heat and mechanical treatment. The resultant nanocomposite materials were collected gently and washed with deionized water and dried in hot air oven at 80 °C for overnight (Bashir, 2022).

Dark brown powdered CuO–melamine nanocomposite obtained as the final product was separated, and this well-ground nanocomposite was stored in air tight containers for morphological, structural and antimicrobial studies. The presence of

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

melamine not only helps in dispersion and stabilization of the structure of the CuO NPs, but it also expands the possibility for application in the field of catalysis, antibacterial and functional coatings (Zhan, 2022).

Table 2.4: Synthesis Parameters for CuO NPs and Nanocomposites

Sample ID	$CuSO_4 \cdot 5H_2O$ Concentration	Melamine Concentration	Volume of Leaf Extract	Temperature	Reaction Time	Observed Color
CuO-2	2 mM (0.318 g)	–	5 mL	80°C	40 min	Yellow-brown
CuO-4	4 mM (0.638 g)	–	5 mL	80°C	40 min	Brown
CuO-6	6 mM (0.957 g)	–	5 mL	80°C	40 min	Dark brown
CM-2	2 mM (0.318 g)	2 mM (0.252 g)	50 mL	80°C	60 min	Dark brown
CM-4	4 mM (0.638 g)	4 mM (0.504 g)	50 mL	80°C	60 min	Dark brown
CM-6	6 mM (0.957 g)	6 mM (0.756 g)	50 mL	80°C	60 min	Dark brown

Characterization Techniques

The as-prepared copper oxide nanoparticles (CuO NPs) and their melamine related nanocomposites were systematically investigated using various physic-chemical techniques for morphological, structural and elemental properties. The used techniques included Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Energy Dispersive X-ray Spectroscopy (EDS/EDX). These methods were complimentary to each other providing complete characterization of physical appearance, particle size and dispersion, crystal structure and purity of the nano-materials (Bushell, 2020).

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

Scanning Electron Microscopy (SEM)

SEM was utilized to investigate the surface morphology, profile, and dispersion of the particles of CuO–melamine nanocomposites and CuO NPs. SEM is used to scan a surface of the sample with a highly focused high-energy electron beam. They strike atoms in specimens and generate secondary electrons, back-scattered electrons, X-rays and other signals that are used to create an image (Hayashi, 2021).

The observations that were made in this report using SEM examination, enabled:

- Shape of the particles (i.e., spherical or rod like) was evaluated by simple observation (i.e. visual analysis) and.
- Evaluation of agglomeration behavior, and
- Quantification of nanoparticle distribution in melamine – evenness and spreading condition (Ouchi, 2020).

Cloud points for low precursor concentrations contain some nanoparticle particles that are larger and more distinct, whereas the cloud points for higher precursor concentration have a denser agglomeration. SEM images also confirmed Mets that melamine was the active surface matrix for CuO NPs both as the supporting material and the morphology stabilizer and dispersion.

Transmission Electron Microscopy (TEM)

While SEM is a surface analyzer, TEM can reveal the internal structure and the nature of the nanocrystal liners of the particle. During transmission electron microscopy (TEM), a super thin sample is irradiated with an electron beam, the transmitted electrons are converged to generate magnified images (Chen, 2020).

The TEM study in the current work provided the following information:

- Size of particle: confirmed to be between 20 and 200 nm depending on the synthesis.
- It goes from round to a little rough around the edges.
- Crystallinity (demonstrated by the resolved lattice fringes in some images (Xiong, 2022).
- Homogeneity, (indicated by homogenous distribution at the lower and slight agglomeration at the higher concentrations).

This kind of HRTEM imaging can be used to verify the successful engineering of

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

CuO at the nanometer scale, co-association with melamine in the composite structures (Zhu, 2022).

Energy Dispersive X-ray Spectroscopy (EDS/EDX):

Elemental compositions of the nanoparticles were analyzed by energy dispersive X-ray spectroscopy (EDS) using the EDAX® AMETEK detector attached to the FEG scanning electron microscopy (FE-SEM).

The EDS to SEM measurement was conducted to determine the elemental analysis and purity of prepared nanoparticles and nanocomposites. Dotting with SEM analysis electron beam causes the emission of from the sample chemical-specific characteristic X-rays. Whereas EDX detects these X-rays and allows for qualitative and quantitative elemental profiles to be generated (Baig, 2024).

The EDX spectrum of the CuO NPs indicated that, the following elements were present:

- Main constituting elements (Cu and O) of the CuO particle. (Mostly derived from the melamine matrix of the nanocomposites).
- Trace elements the 8 samples have complex chemical compositions, including, silicon (Si) and sulfur (S) –may have derived from plant secondary metabolites (phytochemicals) and trace impurities due to laboratory background (Sokan-Adeaga, 2023).

Results and Discussion

Morphological Analysis by SEM

Morphology studies of the prepared CuO nanoparticles as well as melamine nanocomposite have been examined using Scanning Electron Microscope (SEM). SEM images of the CuO NPs prepared at 2, 4, and 6 mM of the $CuSO_4$ concentrations were observed alterations in particle morphology, size, and degree of agglomeration (Bruinink, 2015).

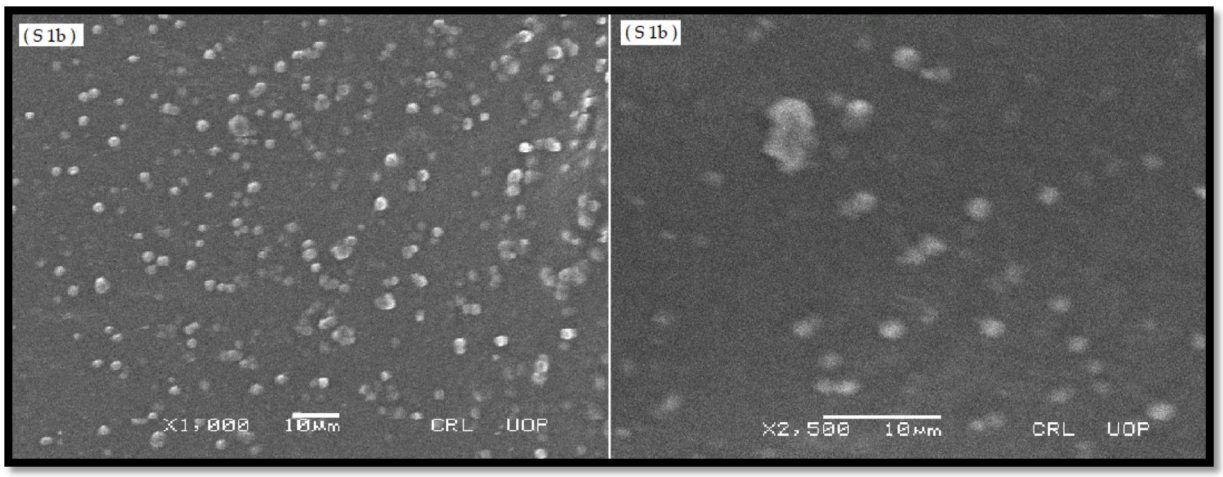
At the concentration of 2 mM, the cuo NPs were spheroidal to prolate-shaped, and they were well-dispersed and slightly aggregating. At 4 mM and 6 mM concentrations, the tubes produced were much more aggregated where individual nanoparticles were less defined, most probably resulting from the possible growing agglomeration due to high ionic interaction and low steric stabilization of the

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

particles (Zhang, 2024).

In contrast, the SEM images of the CuO–melamine nanocomposites indicate that the CuO NPs were well spread on the surface of the melamine matrix, especially at low melamine content 2 mM [br] (Fig. CuO NPs diverged a well dispersed dense surface covering at higher melamine concentrations (4 and 6 mM) as shown in the SEM pictures suggesting that the CuO NPs effectively support the melamine matrix. It is attributed to the strong interaction between the N atoms of melamine and the Cu atoms of the NPs (Franco, 2020).



Fiure 3.1. SEM images of CuO NPS having 02mM salt concentration at 1000 and 2500 resolution

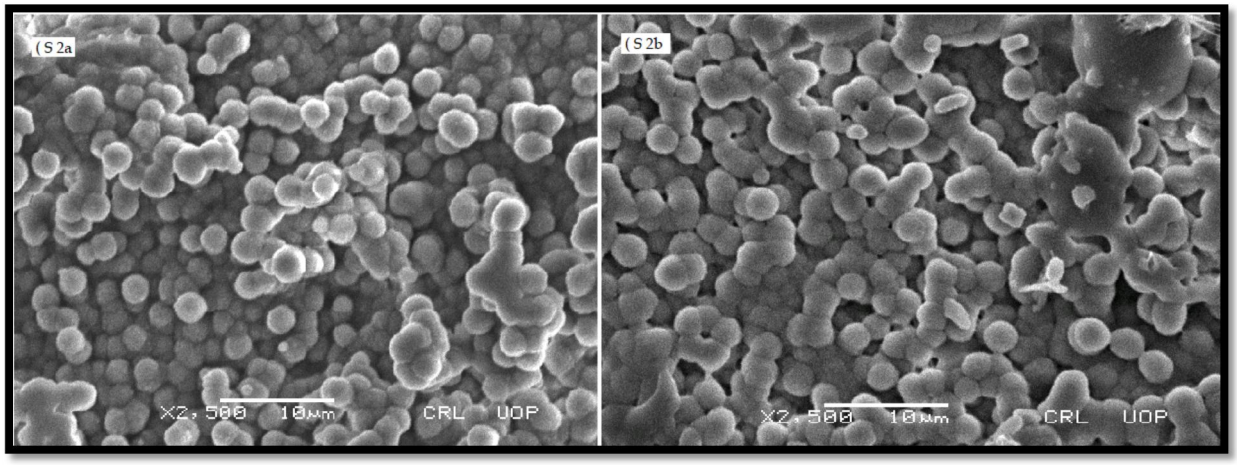


Figure 3.2: SEM images of CuO NPS having 04 mM and salt concentration with (S 2a) X2, 500 and (S2b) with X2, 2500 resolution.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

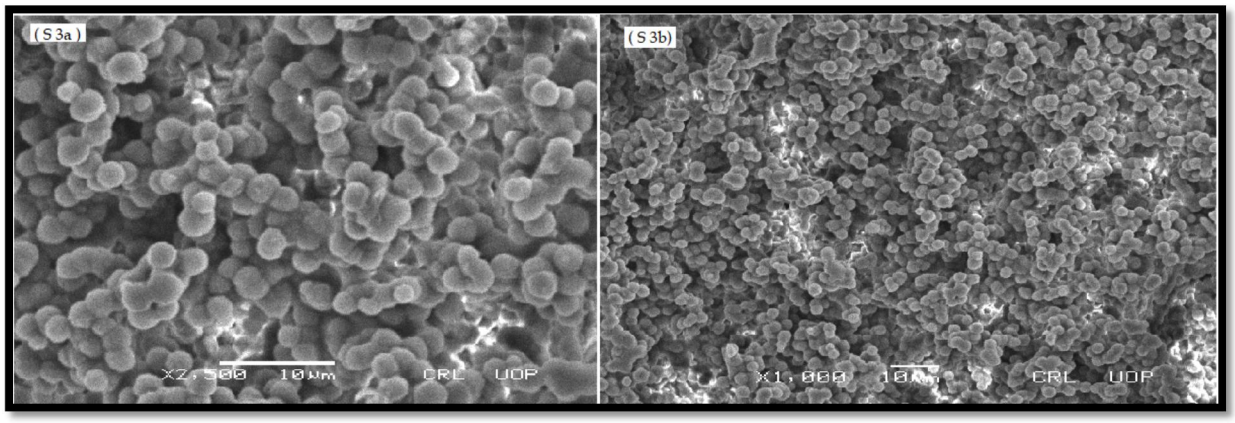


Figure 3.3. SEM images of CuO NPS composite with 06 mM and salt concentration with (S 3a) 500X and(S 3b) 1000X resolution power.

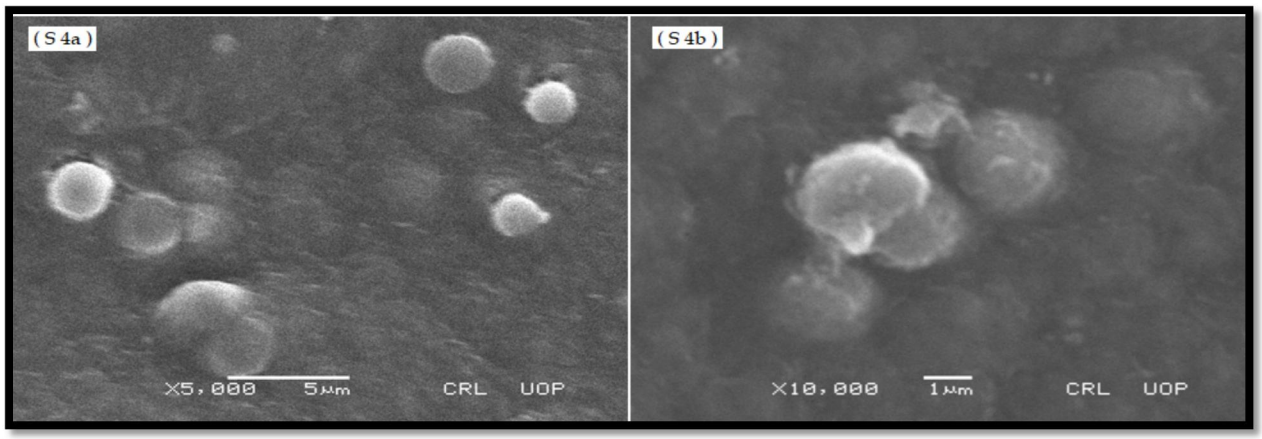


Figure 3.4: SEM Analysis of melamine nanocomposite of02 Mm C3H6N6 at 5000X, 1000X resolution.

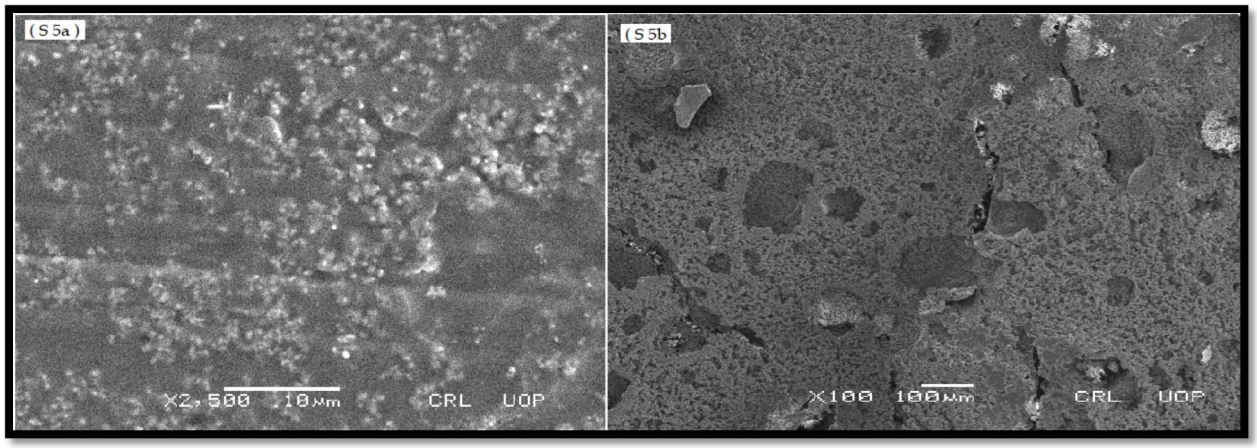


Figure 3.5: SEM Analysis of melamine nanocomposite of 0 Mm C3H6N6 at 2500 X, 100X resolution.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

TEM Analysis of the prepared NPs

The inner morphology and size distribution of the CuO NPs were examined by using TEM (Transmission Electron Microscope). TEM analysis showed that the nanoparticles were of spherical to quasi-spherical shape and the size was in the range of 20–200 nm depending upon the precursor concentration.

The particles were more homogeneous and individual at low salt concentration (2 mM) and more clustered and polydisperse in size at higher salt concentration (6 mM). The edges and the morphologies of the nanoparticles were clear, and their patterns were regular, so it is pretty good evidence for the definite formation of CuO structures. Overall, it is verified that the TEM data and SEM images support and validate the uniform distribution and structural integrity of the synthesized NPs (Hu, 2020).

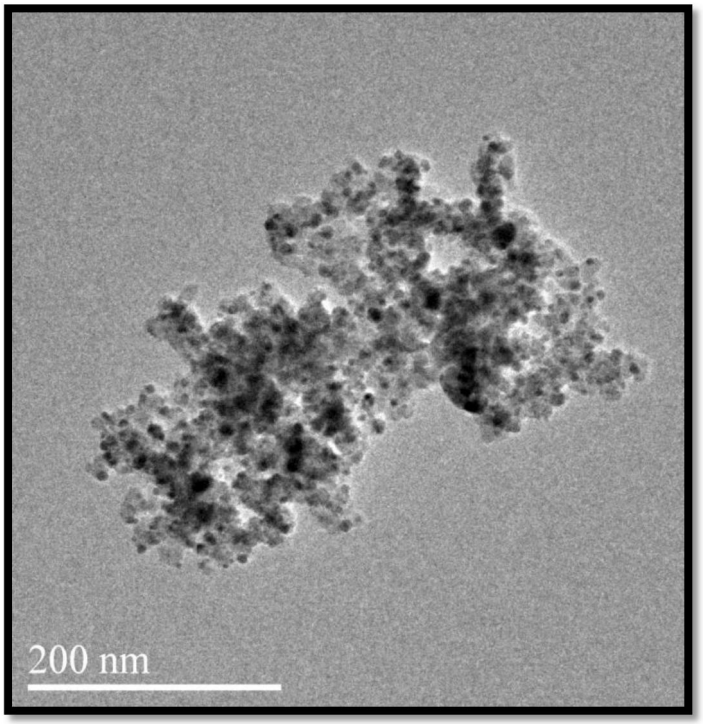


Figure 3.6: TEM images of CuO NPS with 02 mM and salt concentration.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

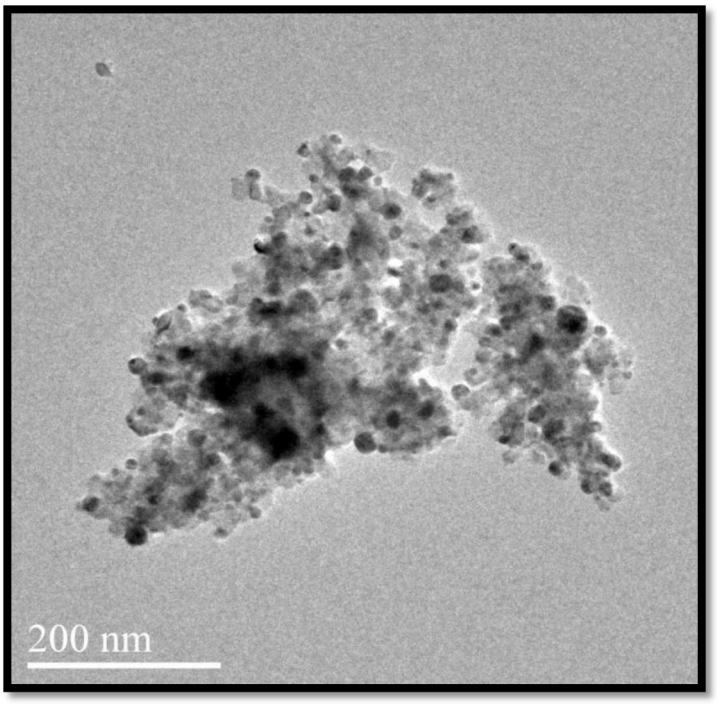


Figure 3.6: TEM images of CuO NPS with 04 mM and salt concentration.

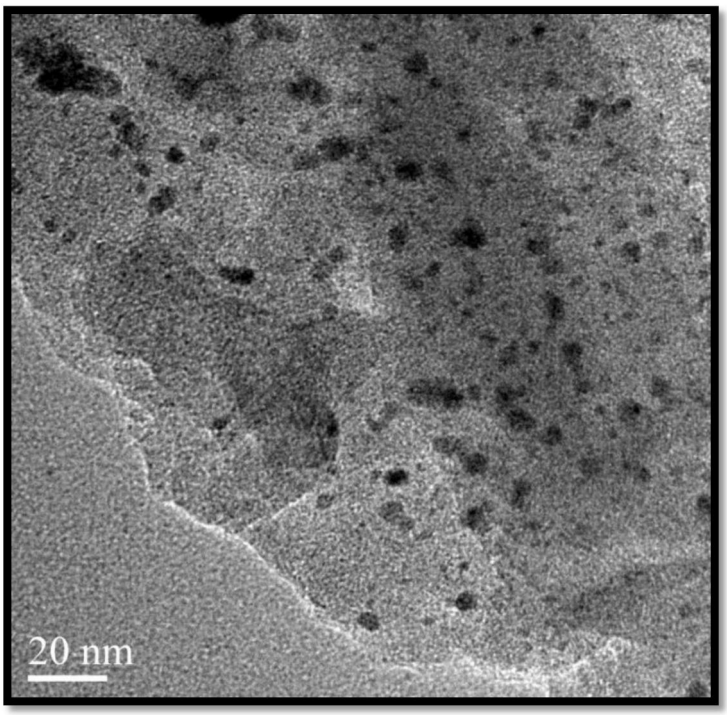


Figure 3.8: TEM images of CuO NPS with 06 mM and salt concentration.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

EDX Analysis The elemental analysis of the samples was performed using EDX

The WT materials were analyzed for their chemical composition with EDX. The peaks were evidence that the primary ingredients, Cu and O existed, and demonstrated that CuO NPs had been successfully prepared. Other elements; carbon (C) and nitrogen (N) were also detected in the nanocomposites and were representative of the presence of melamine; the elements constituting melamine molecule, in the matrix (Hussain, 2020).

Sulphur (S), Si were also found in traces in the system and may be due to either the plant extract or glass wares. More importantly, from the data of the EDX it was found that no trace of pollution was detected, indicating the high purity of the samples. Elemental mapping showed low but homogeneously distributed Cu and O contents, especially at 2 mM salt and the increasing complexed elemental layering in the CuO–melamine composites (Zhen, 2020).

Table 3.1 Elemental compositional Analysis of 04 Mm CuO NPs by EDX Technique

ZzS.No	Elemental composition	Elements Weight %	Elements Atomic weight %
1.	Nitrogen	4.50	4.63
2.	Carbon	43.22	51.87
3.	Oxygen	46.34	41.75
4.	Sulphur	1.26	0.57
5.	Copper	4.25	0.96
6.	Silicone	0.30	0.15
7.	Phosphorous	0.13	0.06
Total		100	100

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

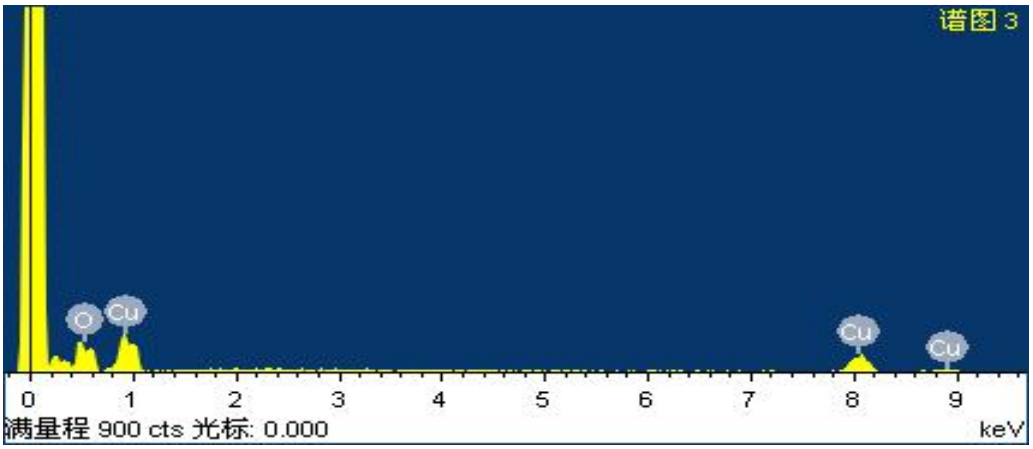


Figure 3.9: EDXAnalysis of 06 Mm of CuO NPs

Antibacterial Assay

The antibacterial activity of the synthesized CuO NPs and CuO–melamine nanocomposites were determined using agar well diffusion assay. The CNSs were tested against six bacteria including Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus, Enterobacter aerogenes, and Bordetella bronchiseptica. References antibiotic drugs -Roxithromycin and Cefixime were used as standard in triplicate (Batista, 2020).

Also, the as-prepared CuO nanoparticles showed moderate antibacterial activities, the diameter of the inhibition zones were in a range of 14–23 mm and these were function of the concentration and the type of the bacteria. The nanocomposites exhibited strong antibacterial activities and the inhibition zones could reach up to 34 mm, in some instances higher than those of standard drug.

This synergism is attributed to the nitrogen-enriched structure of melamine, which could favor the binding to bacterial cell membrane, and thereby, facilitate the cellular uptake of CuO. In addition, increased surface of small NPs size, especially NPs prepared at 2 mM salt concentration, may causing more effective disruption of the membrane and causing more ROS generation and oxygen consumption changes (Sachdev, 2021).

The proposed mechanism involves:

- The electrostatic and oxidative interaction with the bacterial plasma membrane.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

- Accumulation of the cellular Cu^{2+} concurrent with protein and DNA damage.
- Enhanced antimicrobial activity of nanocomposite based on collaborative action of the lone pair electrons of Melamine with bacterial targets (Song, 2024).

Overall, the present study reveals that green synthesize of CuO nanoparticles mediated by plant have shown higher antibacterial activity, which is further enhanced in the form of composites with melamine-based polymer (Alotaibi, 2024).

Table 3.2: Antibacterial Activity of CuO NPs and CuO–Melamine Nanocomposites

Test Organism	CuO-2 (mm)	CuO-4 (mm)	CuO-6 (mm)	CM-2 (mm)	CM-4 (mm)	CM-6 (mm)	Roxythromycin (mm)	Cefixime (mm)
Staphylococcus aureus	16	19	21	25	28	31	23	26
Escherichia coli	14	17	20	24	26	29	21	25
Klebsiella pneumoniae	15	18	22	26	29	32	22	27
Micrococcus luteus	13	15	18	23	26	28	20	24
Enterobacter aerogenes	14	16	19	22	25	30	21	26
Bordetella bronchiseptica	15	17	20	25	28	34	24	28

Conclusion

Copper oxide (CuO) nanoparticles were efficiently synthesized in this work using green and ecofriendly process with S. cumini leaf extract as the biogenic reducing and stabilizing agent. The biosynthesis approach was facile, low-cost and non-toxic and thus offers an environmentally benign option to conventional chemical and physical methods, typically accompanied with harsh conditions and toxic chemicals. The CuO nanoparticles produced by electrospinning were characterized using SEM, TEM, EDX, and they were also found to be spherical in shape with small particles

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

ranging from 20 to 200 nm, and highly pure elements (Kovářík, 2020).

For the enhanced functional effectiveness of the developed NPs, the CuO NPs were confined inside the matrix of the nanocomposite structures using the melamine ($C_3H_6N_6$). SEM, EDX analyses revealed the uniform distribution of the CuO NPs in the melamine matrix because of the presence of nitrogen atoms from the melamine framework. The resulting CuO–melamine nanocomposites exhibited visibly increased antibacterial activity toward the tested bacteria (both Gram-positive and negative bacteria) compared with CuO nanoparticles and even better antibacterial activity compared with some commercial antibiotics. The synergism[13] could be attributed to an addition in physico-chemical bactericidal effect on bacterial cell surface, which enhances ROS generation and membrane damage (Huang, 2020).

Overall, this investigation supports the future predictions of the bio-hybrid CuO NPs and NPs-composite for biomedical and antimicrobial applications. In vivo cytotoxicity of these nanomaterials, their synthesis conditions for large-scale industrial production and their application in drug delivery, water treatment and biosensor development are areas to be investigated in future studies (Elgarahy, 2021).

References

1. Tovar-Lopez, F. J. (2023). Recent progress in micro-and nanotechnology-enabled sensors for biomedical and environmental challenges. *Sensors*, *23*(12), 5406.
2. Altammar, K. A. (2023). A review on nanoparticles: characteristics, synthesis, applications, and challenges. *Frontiers in microbiology*, *14*, 1155622.
3. Oulahal, N., & Degraeve, P. (2022). Phenolic-rich plant extracts with antimicrobial activity: an alternative to food preservatives and biocides?. *Frontiers in Microbiology*, *12*, 753518.
4. Xu, Y. T., Yang, T., Liu, L. L., & Tang, C. H. (2020). One-step fabrication of multifunctional high internal phase pickering emulsion gels solely stabilized by a softer globular protein nanoparticle: S-Ovalbumin. *Journal of colloid and interface science*, *580*, 515-527.
5. Chen, L., Liu, Z., Guo, Z., & Huang, X. J. (2020). Regulation of intrinsic

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

physicochemical properties of metal oxide nanomaterials for energy conversion and environmental detection applications. *Journal of Materials Chemistry A*, *8*(34), 17326-17359.

6. Kumar, A., P, N., Kumar, M., Jose, A., Tomer, V., Oz, E., ... & Oz, F. (2023). Major phytochemicals: recent advances in health benefits and extraction method. *Molecules*, *28*(2), 887.
7. Shah, A., Akhtar, S., Mahmood, F., Urooj, S., Siddique, A. B., Irfan, M. I., ... & Abbas, A. (2024). Fagonia arabica extract-stabilized gold nanoparticles as a highly selective colorimetric nanoprobe for Cd2+ detection and as a potential photocatalytic and antibacterial agent. *Surfaces and Interfaces*, *51*, 104556.
8. Díez-Pascual, A. M. (2020). Antibacterial action of nanoparticle loaded nanocomposites based on graphene and its derivatives: a mini-review. *International Journal of Molecular Sciences*, *21*(10), 3563.
9. Zhang, Y. J., Huang, G. X., Winter, L. R., Chen, J. J., Tian, L., Mei, S. C., ... & Elimelech, M. (2022). Simultaneous nanocatalytic surface activation of pollutants and oxidants for highly efficient water decontamination. *Nature Communications*, *13*(1), 3005.
10. O'Brien, S., Okoffo, E. D., O'Brien, J. W., Ribeiro, F., Wang, X., Wright, S. L., ... & Thomas, K. V. (2020). Airborne emissions of microplastic fibres from domestic laundry dryers. *Science of the Total Environment*, *747*, 141175.
11. Battle, M. W., Vegliani, F., & Jones, M. A. (2020). Shades of green: untying the knots of green photoperception. *Journal of Experimental Botany*, *71*(19), 5764-5770.
12. Chand, K., Cao, D., Fouad, D. E., Shah, A. H., Dayo, A. Q., Zhu, K., ... & Dong, S. (2020). Green synthesis, characterization and photocatalytic application of silver nanoparticles synthesized by various plant extracts. *Arabian Journal of Chemistry*, *13*(11), 8248-8261.
13. Sarkar, J., Chakraborty, N., Chatterjee, A., Bhattacharjee, A., Dasgupta, D., & Acharya, K. (2020). Green synthesized copper oxide nanoparticles ameliorate defence and antioxidant enzymes in Lens culinaris. *Nanomaterials*, *10*(2), 312.
14. Alshehri, S., Imam, S. S., Altamimi, M. A., Hussain, A., Shakeel, F., & Alshehri, A.

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

(2020). Stimulatory effects of Soluplus® on flufenamic acid β-cyclodextrin supramolecular complex: Physicochemical characterization and pre-clinical anti-inflammatory assessment. *AAPS PharmSciTech*, *21*(5), 145.

15. Mohamed, E. A. (2020). Green synthesis of copper & copper oxide nanoparticles using the extract of seedless dates. *Heliyon*, *6*(1).
16. Kim, J., Mayorga-Burrezo, P., Song, S. J., Mayorga-Martinez, C. C., Medina-Sánchez, M., Pané, S., & Pumera, M. (2024). Advanced materials for micro/nanorobotics. *Chemical Society Reviews*.
17. Zhang, S., Huang, Y., Lin, T., Xue, M., Liu, S., Deng, H., & Li, Y. (2025). Novel Cu-based and Pd-based CMC–CA–PVA composite films as efficient dip catalysts for A3 coupling and Suzuki–Miyaura coupling reactions. *Cellulose*, *32*(3), 1645-1665.
18. Bashir, M., Aman, S., Awan, A., Nazar, M. F., Zubair, M., Nadeem, R., & Zafar, M. N. (2022). Metal Sulfide Nanocomposites for Energy Harvesting Applications. In *Advances in Nanocomposite Materials for Environmental and Energy Harvesting Applications* (pp. 567-612). Cham: Springer International Publishing.
19. Zhan, Y., Yu, S., Amirfazli, A., Rahim Siddiqui, A., & Li, W. (2022). Recent advances in antibacterial superhydrophobic coatings. *Advanced Engineering Materials*, *24*(4), 2101053.
20. Bushell, M., Beauchemin, S., Kunc, F., Gardner, D., Ovens, J., Toll, F., ... & Johnston, L. J. (2020). Characterization of commercial metal oxide nanomaterials: crystalline phase, particle size and specific surface area. *Nanomaterials*, *10*(9), 1812.
21. Hayashi, H., Kimoto, N., Asahara, T., Asakawa, T., Lee, C., & Katsumata, A. (2021). Photon counting detectors for x-ray imaging. *Springer International Publishing. doi*, *10*, 978-3.
22. Ouchi, S., Yamada, N., & Yamamoto, T. (2020). Size control of polystyrene nanoparticles synthesized in melamine foam. *Industrial & Engineering Chemistry Research*, *59*(40), 17927-17933.
23. Chen, Q., Dwyer, C., Sheng, G., Zhu, C., Li, X., Zheng, C., & Zhu, Y. (2020). Imaging beam-sensitive materials by electron microscopy. *Advanced*

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

Materials, *32*(16), 1907619.

24. Xiong, Z., Sun, B., Zou, H., Wang, R., Fang, Q., Zhang, Z., & Qiu, S. (2022). Amorphous-to-crystalline transformation: general synthesis of hollow structured covalent organic frameworks with high crystallinity. *Journal of the American Chemical Society*, *144*(14), 6583-6593.
25. Zhu, Z., Wei, C., Jiang, D., Wu, X., & Lu, M. (2022). Design and synthesis of MOF-
26. Baig, M. A., Fayyaz, A., Ahmed, R., Umar, Z. A., Asghar, H., Liaqat, U., ... & Kurniawan, K. H. (2024). Analytical techniques for elemental analysis: LIBS, LA-TOF-MS, edx, pixe, and XRF: a review. *Proceedings of the Pakistan Academy of Sciences: A. Physical and Computational Sciences*, *61*(2), 99-112.
27. Sokan-Adeaga, A. A., Sokan-Adeaga, M. A., Sokan-Adeaga, E. D., Oparaji, A. N., Edris, H., Tella, E. O., ... & Amubieya, O. E. (2023). Environmental toxicants and health adversities: A review on interventions of phytochemicals. *Journal of public health research*, *12*(2), 22799036231181226.
28. Bruinink, A., Wang, J., & Wick, P. (2015). Effect of particle agglomeration in nanotoxicology. *Archives of toxicology*, *89*(5), 659-675.
29. Zhang, H., Du, S., Wang, Y., & Xue, F. (2024). Prevention of crystal agglomeration: Mechanisms, factors, and impact of additives. *Crystals*, *14*(8), 676.
30. Franco, F., Rettenmaier, C., Jeon, H. S., & Cuenya, B. R. (2020). Transition metal-based catalysts for the electrochemical CO 2 reduction: from atoms and molecules to nanostructured materials. *Chemical Society Reviews*, *49*(19), 6884-6946.
31. Hu, Q., Hu, S., Fleming, E., Lee, J. Y., & Luo, Y. (2020). Chitosan-caseinate-dextran ternary complex nanoparticles for potential oral delivery of astaxanthin with significantly improved bioactivity. *International journal of biological macromolecules*, *151*, 747-756.
32. Hussain, I., Qi, J., Sun, X., Wang, L., & Li, J. (2020). Melamine derived nitrogen-doped carbon sheet for the efficient removal of chromium (VI).
33. Zhen, H. G., Mao, H., Haq, I. U., Li, S. H., Ahmad, A., & Zhao, Z. P. (2020). In-situ SIFSIX-3-Cu growth into melamine formaldehyde sponge monolith for CO2

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

efficient capture. *Separation and Purification Technology*, *233*, 116042.

34. Batista, A. D., A. Rodrigues, D., Figueiras, A., Zapata-Cachafeiro, M., Roque, F., & Herdeiro, M. T. (2020). Antibiotic dispensation without a prescription worldwide: a systematic review. *Antibiotics*, *9*(11), 786.
35. Sachdev, S., Ansari, S. A., Ansari, M. I., Fujita, M., & Hasanuzzaman, M. (2021). Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. *Antioxidants*, *10*(2), 277.
36. Song, H., Zhang, K., Li, P., Qin, G., Xiao, W., Zhang, C., ... & Ji, S. (2024). One-step construction of silver–polyaniline nanocomposite modified multifunctional sponges for wastewater remediation: adsorption, catalysis and antimicrobial applications. *Journal of Materials Chemistry A*, *12*(11), 6747-6767.
37. Alotaibi, M. M., Almalki, B., Tashkandi, N., Basingab, F., Abdullah, S., & Alkayal, N. S. (2024). Synthesis of silver nanoparticles embedded into melamine polyaminal networks as antibacterial and anticancer active agents. *Scientific Reports*, *14*(1), 20008.
38. Kovářík, T., Bělský, P., Rieger, D., Ilavsky, J., Jandová, V., Maas, M., ... & Medlín, R. (2020). Particle size analysis and characterization of nanodiamond dispersions in water and dimethylformamide by various scattering and diffraction methods. *Journal of Nanoparticle Research*, *22*(2), 34.
39. Huang, S., Song, Q., Li, Q., Zhang, H., Luo, X., & Zheng, Z. (2020). Damage of heavy metals to Vallisneria natans (V. natans) and characterization of microbial community in biofilm. *Aquatic Toxicology*, *225*, 105515.
40. Elgarahy, A. M., Elwakeel, K. Z., Akhdhar, A., & Hamza, M. F. (2021). Recent advances in greenly synthesized nanoengineered materials for water/wastewater remediation: an overview. *Nanotechnology for Environmental Engineering*, *6*(1), 9.