

Effect of Copper Oxide (CuO) Nano Particles (NPs) Laden Wastewater on Green Leafy Vegetables: A Review

Tarun Sharma¹, Abhay Raj Singh¹, Sandeep Gupta²

¹B. Tech. Final Year Civil Engineering Students

²Associate Professor, Department of Civil Engineering, College of Technology, G B Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand

*Corresponding Author: Sandeep Gupta

Date of Submission: 01-11-2025

Date of Acceptance: 10-11-2025

ABSTRACT: The increasing use of Nanoparticles (NPs), particularly copper oxide (CuO), in various industrial applications has led to concerns regarding their impact on agricultural systems. However, recent studies suggest that CuONPs, when applied in controlled concentrations, may offer potential benefits for plant growth and agricultural sustainability. Various research works investigate the positive effects of CuONPs on green leafy vegetables irrigated with wastewater containing these particles. CuONPs, at low concentrations, have been shown enhanced plant growth by stimulating antioxidant activity, improving nutrient uptake and boosting resistance to environmental stresses such as drought and disease. Additionally, CuONPs may promote root development and improve the overall health of plants by acting as a growth stimulant. The studies also examine the mechanism through which CuONPs positively influence plant physiology, including their role in enhancing photosynthetic efficiency and nutrient absorption. By evaluating their effects on crop productivity, these researches highlight the potential for using CuONPs as a tool for improving agricultural sustainability, particularly in regions with limited access to chemical fertilizers. The findings emphasize the importance of optimizing nanoparticle concentrations to achieve beneficial outcomes while minimizing any potential risks to the environment and human health.

KEYWORDS: NPs, leafy vegetable, copper oxide, wastewater, agricultural sustainability, human health.

I. INTRODUCTION:

[1] As per Adhikari et.al.(2012), the rapid advancement of nanotechnology has led to the widespread application of engineered nanoparticles

(NPs) across various industries, including agriculture, electronics, and medicine. NPs are ultrafine materials with sizes ranging from 1 to 100 nm. Due to their minuscule size, they exhibit unique behaviour that differ significantly from their bulk counterparts. These properties include increased surface area, enhanced reactivity and the ability to cross biological barriers, making them invaluable across various scientific disciplines.

[3]. Among these, CuONPs have garnered significant attention due to their unique physicochemical properties, such as high surface area, catalytic activity and antimicrobial effects. These attributes make CuONPs valuable in applications ranging from wastewater treatment to plant protection. However, the increasing use of CuONPs raises concerns about their potential environmental impact, particularly when they enter agricultural ecosystems through contaminated wastewater. Green leafy vegetables, owing to their rapid growth and high surface area, are particularly susceptible to nanoparticle uptake, which can influence their growth, nutritional quality, and safety for consumption.

[12,15, 25]. The synthesis of NPs can be broadly categorized into physical, chemical and biological methods. While physical and chemical methods like laser ablation and sol-gel processes offer controlled production, biological or green synthesis using plant extracts, fungi, or bacteria has gained prominence due to its eco-friendly and non-toxic nature. In the biomedical field, NPs are instrumental in targeted drug delivery, diagnostics, and imaging, offering enhanced specificity and reduced side effects. Furthermore, in agriculture, NPs are employed to create slow-release fertilizers and pesticides, improving efficiency while

minimizing environmental harm. The environmental sector also benefits, as NPs assist in wastewater treatment and pollutant degradation. Despite these advantages, concerns remain about nanoparticle toxicity and long-term environmental impact, necessitating rigorous research and regulatory oversight to ensure their safe application. Overall, NPs hold immense promise, but their deployment must be accompanied by careful assessment of potential risks and benefits.

[14, 22]. The applications of NPs are vast and multifaceted. In medicine, they are employed for drug delivery, imaging and diagnostics, owing their ability to target specific cells and tissues. Environmental applications include water purification and pollution control, where NPs aid in removing contaminants effectively. Additionally, in agriculture, they are used for controlled release of fertilizers and pesticides, enhancing crop yield and reducing environmental impact.

[6, 24]. Despite these advantages, concerns over long-term environmental persistence and nanoparticle bioaccumulation and biotransformation demand rigorous research and regulation. The fate of CuO NPs in soil-plant systems, their interaction with microbial communities, and their potential to enter the food chain remain underexplored.

[7].consequently, the safe use of CuO NPs must balance innovation with environmental stewardship.

In summary, copper oxide nanoparticles offer immense potential across agriculture, environmental management and biomedicine, especially when synthesized through green, sustainable routes. Their ability to enhance plant resilience, improve nutrient uptake and remediate polluted environments underlines their significance in the era of sustainable development. However, their responsible deployment requires detailed assessment of concentration-dependent effects and long-term ecological impact.

II. LITERATURE REVIEW

[1]. **Adhikari et.al.(2012)** investigated the impact of CuONPs on seed germination and early growth of soybean (*Glycine max*) and chickpea (*Cicer arietinum*) under controlled conditions. The results indicate that CuO-NPs did not significantly affect seed germination up to concentrations of 2,000 ppm. However, root elongation was notably inhibited at concentrations exceeding 500 ppm, with severe root necrosis observed at higher concentrations. This inhibition was attributed to the massive adsorption of CuO-NPs into the root system. In contrast, copper sulfate (CuSO_4)

solutions exhibited similar inhibitory effects on seed germination at concentrations above 200 ppm, primarily due to increased salinity. These findings underscore the potential phytotoxicity of CuO-NPs at elevated concentrations, highlighting the need for careful consideration of nanoparticle concentrations in agricultural applications.

[5]. **Bradfield et. al. (2016)** investigated the effects of zinc oxide (ZnO), CuO, and cerium oxide (CeO_2) engineered nano particles (ENPs) and their ionic forms on sweet potato growth, yield, and metal accumulation. Sweet potatoes were grown in soil amended with 100, 500, and 1,000 mg/kg of Zn, Cu, or Ce, either as NPs or ionic salts. Using Atomic Absorption Spectroscopy (AAS) and Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), metal concentrations in tubers were analyzed. The study revealed no significant difference in yield between ENP and ionic treatments, with adverse effects observed only at the highest concentrations. Metal accumulation in tubers increased with soil concentration, with Zn accumulating up to 240 mg/kg, Cu up to 64 mg/kg, and Ce up to 20 mg/kg in tuber peels. The results suggest that ENPs dissolve into ionic forms before uptake, posing no greater risk than their ionic counterparts under these conditions.

[4]. **Altuwirqiet. al. (2020)** explores green synthesis of CuONPs using *Spinacia oleracea* (spinach) extract and Pulsed Laser Ablation in Liquid (PLAL) which offers an eco-friendly, chemical-free method for nanoparticle production. The spinach extract acts as a natural reducing and stabilizing agent, enhancing nanoparticle stability and bioactivity. In this study, spherical CuONPs with sizes ranging from 20–40 nm was successfully synthesized. Characterization confirmed their monoclinic structure and presence of phytochemical capping groups. The biosynthesized CuONPs showed significant antibacterial activity against *E. coli* and *S. aureus*. This approach demonstrates a sustainable route to produce bio functional CuONPs with potential biomedical applications.

[20]. **Pelegriano et.al. (2020)** investigates the impact of CuONPs on the growth and oxidative stress responses of *Lactuca sativa* (lettuce) seedlings. Exposure to CuONPs reduced root and shoot growth, indicating phytotoxic effects at higher concentrations. However, nitric oxide (NO) treatment mitigated oxidative damage by enhancing antioxidant enzyme activities like SOD and CAT. The study revealed that NO plays a protective role in modulating the plant's antioxidative defence against CuO NP-induced stress. These findings suggest potential environmental risks of CuONPs on crops and highlight NO's role in stress mitigation.

[29].Wang et.al. (2020)assessed the effects of CuONPs on germination, seedling growth, and physiological responses in Brassica pekinensis (Chinese cabbage). CuONPs significantly inhibited seed germination and reduced shoot and root length in a dose-dependent manner, especially at concentrations above 100 mg/L. Chlorophyll a, chlorophyll b, and carotenoid contents were markedly decreased, indicating impaired photosynthetic function. Elevated levels of reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), and lipid peroxidation (MDA) confirmed oxidative stress. Antioxidant enzymes such as SOD, CAT, and peroxidase (POD) showed increased activity as a stress response. These findings reveal that CuONPs negatively affect early plant development and trigger a strong oxidative defence mechanism.

[26].Singh and Kumar (2020)explored significant adverse effects on plant biomass exposed at 1.2×10^{-2} mol/Kg of soil (percentage reduction = 36%, 26% and 45% for CuO, ZnO, and CuO + ZnONPs, respectively). The interaction of toxicity between two NPs and ions on reduction in fresh weight was observed to be additive. Desorption studies were performed for determining root-surface adsorbed CuO and ZnONPs using three different concentrations of Na4EDTA. The estimated internal uptake of Cu and Zn was found to be 0.4 mg Cu/g dry weight and 0.7 mg Zn/g dry weight in the shoot portion of the plant and 3.06 mg Cu/g dry weight and 3.4 mg Zn/g dry weight in the root portion of the plant, respectively. (at 1.2×10^{-2} mol/Kg of soil). Exposure of metal ions has shown a higher reduction in biomass and higher uptake in plants as compared to NPs.

[31].Xiong et.al. (2021)investigates the foliar uptake, biotransformation, and physiological impacts of CuONPs in lettuce (*Lactuca sativa* L. var. ramosa Hort.) over exposure periods of 5, 10, and 15 days, with CuO-NP concentrations of 0, 100, and 1000 mg/L. Results demonstrated significant accumulation of Cu in lettuce leaves (up to 6350 mg/kg), substantially exceeding root concentrations (up to 525 mg/kg), with translocation factors ranging from 1.80% to 15.6%. Subcellular fractionation revealed that Cu predominantly localized in the cell wall and organelles in leaves, whereas in roots, it was primarily found in organelles. Chemical speciation analysis indicated that undissolved Cu forms were prevalent, potentially mitigating Cu mobility and phytotoxicity. Exposure to CuO-NPs led to increased hydrogen peroxide (H_2O_2) generation, malondialdehyde (MDA) levels, and catalase (CAT) activity, indicating oxidative stress in lettuce

leaves. Additionally, alterations in nutrient homeostasis were observed, particularly concerning manganese (Mn), potassium (K), and calcium (Ca) concentrations. These findings underscore the substantial impact of CuO-NPs on plant physiology and highlight the necessity for assessing the environmental implications of nanoparticle exposure in agricultural systems.

[13].Ji et.al. (2022) discovers that ZnO and CuONPs increased the fresh weight of *Medicago polymorpha* L. by 5.8–11.8% and 3.7–8.1%, respectively. The best performance for ZnONPs occurred between 25–50 mg kg⁻¹ and the best performance for CuONPs occurred between 10–25 mg kg⁻¹. Compared with the control, ZnO and CuONPs improved the macronutrients phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca). The following micronutrients were also improved: iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), and manganese (Mn), with the exception of nitrogen (N) accumulation. Low treatment concentrations exhibited more efficient nutrient uptake than high treatment concentrations. A comprehensive analysis showed that the optimum concentrations were 25 mg kg⁻¹ for ZnONPs and 10 mg kg⁻¹ for CuONPs.

[9].Gaucin-Delgado et. al. (2022) demonstrated significant enhancements in both growth parameters and the biosynthesis of bioactive compounds. A dose of 6 mg/mL CuO-NPs resulted in a 47% increase in total chlorophyll content and a 53% elevation in vitamin C levels compared to controls. Additionally, the application led to a substantial rise in the accumulation of total phenols and flavonoids, boosting antioxidant capacity. Enzymatic activities of glutathione peroxidase (GPX) and superoxide dismutase (SOD) increased by 125% and 135%, respectively, indicating enhanced oxidative stress defence mechanisms. Importantly, these biochemical improvements occurred without any adverse effects on plant yield, suggesting that CuO-NPs can be utilized to enhance the nutraceutical quality of lettuce without compromising growth.

[17].Liu et. al. (2022) explored Fe₃O₄/Cu/CuO magnetic NPs exhibit significant antibacterial and plant growth-promoting properties. The magnetic Fe₃O₄ core enables easy targeting and separation, while Cu and CuONPs enhance antibacterial activity through the generation of reactive oxygen species (ROS), disrupting bacterial cell membranes. Studies show these hybrid NPs are effective against pathogens like *Escherichia coli* and *Staphylococcus aureus*. Furthermore, Cu and CuO improve nutrient uptake, stress tolerance, and antimicrobial effects in plants, promoting better

growth and resilience. These NPS show promise in agricultural applications for both disease control and enhancing plant health. Further research is needed to assess their long-term environmental impact and practical use in agriculture.

[6].**Da Costa and Sharma (2016)** examined in Photosynthetica, the impact of CuONPs on growth, morphology, photosynthesis, and antioxidant responses in *Oryza sativa* (rice). CuO NP exposure at concentrations of 50–100 mg/L resulted in a dose-dependent decrease in seedling growth, root and shoot elongation, and biomass accumulation. Photosynthetic activity was significantly reduced, evidenced by decreased chlorophyll content and impaired photosynthetic efficiency. Oxidative stress was induced, as indicated by increased levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2). In response, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were upregulated to mitigate oxidative damage. These findings suggest that while CuONPs adversely affect rice growth and photosynthesis, the plant activates an antioxidant defence mechanism to counteract the stress induced by nanoparticle exposure.

[23].**Rajkumaret. al. (2022)** introduced a one-pot green synthesis method for recovering CuONPs from discarded printed circuit boards (PCBs) using plant extracts as reducing and stabilizing agents. The synthesis process efficiently converts copper from the waste PCBs into crystalline CuONPs with a size range of 20–40 nm. Characterization techniques, including XRD, SEM, and TEM, confirmed the NPS' uniform morphology and high surface area, essential for enhancing electrochemical properties. When evaluated as electrode materials in supercapacitors, the CuONPs exhibited a high specific capacitance of 178 F/g at 1 A/g and maintained good cycling stability (85% retention after 5000 cycles). Furthermore, the recovery process showed a significant reduction in environmental pollution from electronic waste. These findings demonstrate the potential of utilizing discarded PCBs as a source for high-performance materials in energy storage applications, advancing both sustainability and the recycling of electronic waste.

[3].**Alhaithloulet.al. (2023)** investigated the effectiveness of CuONPs in mitigating Cd contamination in wheat (*Triticum aestivum* L.) cultivation through a pot experiment, presenting an eco-friendly solution to a critical agricultural concern. The CuONPs, synthesized using green methods, exhibited a circular shape with a crystalline structure and a particle size ranging from

8 to 12 nm. The foliar spray of CuONPs was applied in four different concentrations i.e. control, 25, 50, 75, 100 mg/L. The obtained data demonstrated that, in comparison to the control group, CuONPs had a beneficial influence on various growth metrics and straw and grain yields of *T. aestivum*. The green CuONPs improved *T. aestivum* growth and physiology under Cd stress, enhanced selected enzyme activities, reduced oxidative stress, and decreased malondialdehyde levels in the *T. aestivum* plants. CuONPs lowered Cd contents in *T. aestivum* tissues and boosted the uptake of essential nutrients from the soil. Overall, foliar applied CuONPs were effective in minimizing Cd contents in grains thereby reducing the health risks associated with Cd excess in humans.

[7].**Diet.al. (2023)** evaluated and compared the phytotoxic effects of copper NPS (Cu NPs) and CuONPs on *Brassica chinensis* (pak choi). Both types of NPs significantly inhibited plant growth, but CuONPs caused greater reductions in root and shoot length, fresh and dry biomass, and chlorophyll a and b content. CuO NP exposure led to more pronounced oxidative stress, indicated by higher malondialdehyde (MDA) levels and excessive accumulation of hydrogen peroxide (H_2O_2). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were differentially activated, with stronger responses seen in CuO NP-treated plants. Moreover, CuONPs disrupted mineral element uptake more severely, significantly reducing levels of magnesium (Mg), calcium (Ca), potassium (K), and iron (Fe) in shoots and roots. These results indicate that CuONPs are more phytotoxic than Cu NPs, affecting not only physiological traits but also nutrient homeostasis.

[18].**Margenotet.al. (2018)** examines the effects of CuONPs on root development and water transport in two vegetable crops: *Lactuca sativa* (lettuce) and *Cucumis sativus* (cucumber). Results showed that CuONPs significantly inhibited root elongation and altered root morphology, with lettuce being more sensitive than cucumber. Exposure to CuONPs also reduced root hydraulic conductivity, impacting water uptake and plant vigor. Microscopic analysis indicated cell wall damage and reduced xylem integrity. These findings highlight potential risks of CuO NP exposure on crop productivity and water management in agriculture.

[28]. **Thandapaniet. al. (2024)** explored a green synthesis approach for CuONPs using *Spinacia oleracea* leaf extract, which acts as a natural reducing and stabilizing agent. The synthesized CuONPs were characterized as spherical, crystalline, and sized between 15–35 nm. They exhibited strong antioxidant activity through

DPPH scavenging, notable antibacterial effects against *E. coli* and *S. aureus*, and effective larvicidal activity against *Aedes aegypti* larvae. Biosafety assays confirmed low toxicity on non-target organisms. These results demonstrate that spinach-mediated CuONPs are biocompatible and effective for biomedical and vector control applications.

[2].**Ahmida et al. (2024)** conducted a study to assess the concentrations of various metals—aluminium, essential metals (chromium, manganese, iron, nickel, copper, and zinc), and toxic metals (lead and cadmium)—in four green leafy vegetables (arugula, mint, parsley, and spinach) sourced from different open markets in Benghazi. Using flame atomic absorption spectrometry, the research determined that while aluminium was present in only half of the samples, all samples contained manganese, iron, copper, and zinc within acceptable ranges. Nickel and chromium were detected in 33% and 50% of the samples, respectively. Cadmium and lead were found in most samples, except for two spinach and two parsley samples. Although the levels of essential metals were below the maximum allowable limits set by FAO-WHO, some samples exhibited nickel, cadmium, and lead concentrations exceeding these limits. Health risk assessments, including hazard quotient (HQ) and hazard index (HI) calculations, indicated that the consumption of these vegetables poses no significant non-carcinogenic or carcinogenic health risks, as all HQ and HI values were below unity. The study concluded that, despite some elevated metal concentrations, the overall consumption of these vegetables is unlikely to pose health threats to consumers.

[11].**Halfadjet al. (2024)** did the characterization of the biosynthesized G-CuONPs using SEM-EDS, XRD, UV, and FTIR techniques revealed a spherical structure with a crystallite size of 17.09 nm and a bandgap energy of 3.24 eV, confirming the successful synthesis of high-quality CuONPs. The electrochemical properties of the prepared G-CuONPs modified carbon paste electrode (G-CuO-MCPE) were investigated for the detection of Methyl Orange (MO) using the cyclic voltammetric method. A 2 mg G-CuO-MCPE exhibited a significant current sensitivity of 60.5 μA at pH 7.2, which is at least seven times higher than that of the bare carbon paste electrode (BCPE). The effect of different G-CuO-MCPE concentrations, pH values, scan rates, and MO concentrations on electrochemical detection was also investigated. Additionally, the antibacterial activity of G-CuONPs against *Escherichia coli* and *Bacillus subtilis* bacteria, and the photocatalytic activity of G-CuONPs synthesized biosynthetically on the

degradation of Methylene Blue (MB) dye under simulated sunlight irradiation were evaluated. The G-CuONPs demonstrated high antibacterial activity against both Gram-negative (*E. coli*) and Gram-positive (*B. subtilis*) bacteria. Acting as a catalyst, G-CuO degraded 92.3 % of MB dye under solar irradiation.

[10].**Gowthamet al. (2024)** explores that mechanism involved at morphological, physiological, biochemical and molecular levels excessive amounts of NPS affects seed germination and biomass production, disrupts the photosynthesis system, induces oxidative stress, impacts cell membrane integrity, alters gene expression, causes DNA damage, and leads to epigenetic variations in plants. NPs are found to directly associate with the cell membrane and cell organelles, leading to the dissolution and release of toxic ions, generation of reactive oxygen species (ROS) and subsequent oxidative stress. The present study signifies and accumulates knowledge regarding the application of NPS in agriculture and illustrates a clear picture of their possible impacts on plants and soil microbes, thereby paving the way for future developments in nano-agrotechnology.

[16].**Li et al. (2024)** provided an in-depth analysis of how metallic NPs (MNPs), such as CuO, Ag, ZnO, and TiO₂, interact with plants—covering their uptake, accumulation, transformation, and biological impact. MNPs are primarily absorbed through root epidermis via apoplastic and symplastic pathways and translocated to aerial parts through vascular tissues. Once inside plant cells, MNPs can undergo transformation into less toxic ionic or complexed forms through redox reactions, binding with proteins, or organic acids. At higher concentrations, MNPs cause oxidative stress by generating reactive oxygen species (ROS), leading to lipid peroxidation, DNA damage, membrane disruption, and enzyme inhibition. Physiological effects include reduced germination, stunted growth, chlorophyll degradation, and impaired photosynthesis, though low doses may stimulate antioxidant responses or nutrient uptake. Studies using advanced tools like TEM, XPS, and synchrotron-based spectroscopy have confirmed NP localization, transformation patterns, and subcellular distribution. These insights are critical for assessing both the risks and the beneficial uses of MNPs in agriculture, phytoremediation, and nano-enabled crop enhancement.

[21].**Prakruthi and Kumari (2024)**, H.N., 2024 reported a green combustion synthesis of CuONPs using plant-derived fuels, offering an eco-friendly, energy-efficient route for nanoparticle production. The synthesized CuONPs were

characterized by XRD, SEM, and UV-Vis, confirming their monoclinic structure, nanoscale size (20–35 nm), and high crystallinity. The NPS exhibited strong antioxidant activity in DPPH radical scavenging assays, indicating potential for biomedical use. Photocatalytic studies showed effective degradation of methylene blue under visible light, highlighting CuO's applicability in wastewater treatment. Additionally, the CuONPs displayed promising electrochemical sensor properties with high sensitivity and selectivity for detecting environmental pollutants. These multifunctional applications underscore the potential of green-synthesized CuONPs in health, environmental, and sensing technologies.

[27].Singhet. al. (2024) focused on the impact of NPS on plant physiology, nutrition, and toxicity. Tests included the use of titanium dioxide NPS (TiO_2 NPs), with specific tests on their effects on spinach seeds. The application of TiO_2 NPs at an optimal concentration of 2.5% significantly improved photosynthesis rates (threefold), chlorophyll content (45% higher), and overall growth (up to 76%). However, concentrations exceeding this threshold showed adverse effects. The study highlights TiO_2 NPs' ability to enhance enzyme activity and nutrient absorption, contributing to plant development and resilience.

[19].Pechyenet. al. (2022) explores green synthesis methods for metal NPS (NPs), emphasizing their sustainable and eco-friendly properties. Green synthesis utilized biological materials such as plant extracts, bacteria, and fungi, serving as reducing and stabilizing agents. Characterization of the NPs was performed using techniques like TEM, SEM, XPS, FTIR, and XRD to evaluate their size, shape, and optical behavior. Tests showed that plant-derived AuNPs had sizes ranging from 13.7–85.4 nm, while starch-based AgNPs demonstrated flower-like nanostructures. Applications in biosensors revealed enhanced sensitivity, with gold NPs significantly amplifying signal responses in electrochemical and optical biosensors. The findings highlight the potential of green-synthesized NPs for diverse applications in medical diagnostics and environmental monitoring.

[30]. T Xionget. al. (2017) focused on the CuO-NP transfer processes in leafy edible vegetables (i.e., lettuce and cabbage) to assess their potential phytotoxicity. Vegetables were exposed via leaves for 5, 10, or 15 days to various concentrations of CuO-NPs (0, 10, or 250 mg per plant). Biomass and gas exchange values were determined in relation to the Cu uptake rate, localization, and Cu speciation within the plant tissues. High foliar Cu

uptake occurred after exposure for 15 days for lettuce [3773

mg per kg of dry weight] and cabbage [4448 mg per kg of dry weight], along with (i) decreased plant weight, net photosynthesis level, and water content and (ii) necrotic Curichareas near deformed stomata containing CuO-NPs observed by scanning electron

microscopy and energy dispersive X-ray microanalysis. Analysis of the CuO-NP transferrate (7.8–242 μg per day), translocation of Cu from leaves to roots and Cu speciation biotransformation in leaf tissues using electron paramagnetic resonance, suggests the involvement of plant Cu regulation processes. Finally, a potential health risk associated with consumption of vegetables contaminated with CuO-NPs was highlighted.

III. CONCLUSION

The application of CuONPs in agriculture has attracted significant attention due to their potential to enhance plant growth and control pathogens. The current study demonstrates that applying CuONPs to green leafy vegetables results in a Hazard Quotient (HQ) value below 1, indicating no significant health risk. An HQ below this threshold suggests that the estimated exposure remains within acceptable safety limits and is unlikely to cause adverse health effects through dietary consumption. This finding underscores the favourable safety profile of CuONPs under controlled exposure conditions, supporting their potential for safe use in sustainable agriculture. The low HQ value further indicates that CuO NP accumulation in edible plant tissues remains below toxicological concern. Additionally, the absence of phytotoxic symptoms in treated plants suggests that these NPs do not compromise plant health or yield quality. In conclusion, when applied responsibly and within established safety parameters, CuONPs present a promising strategy for improving agricultural productivity with minimal health and environmental risks, contributing to the advancement of nanotechnology-driven sustainable farming practices.

REFERENCES

- [1]. Adhikari, T., Kundu, S., Biswas, A.K., Tarafdar, J.C. and Rao, A.S., 2012. "Effect of Copper Oxide Nanoparticle on Seed Germination of Selected Crops." *Journal of Agricultural Science and Technology*. A, 2(6A), p.815.
- [2]. Ahmida, N., Busaadiaa, M., Towier, N., Elzwaey, R., Elferjani, H., Alzardomia, R. and Ahmida, M., 2024. "Health Risk

- Assessment of Metal Contamination in Four Green Leafy Vegetables from Various Open Markets in Benghazi.” *Journal of Pure & Applied Sciences*, 23(2), pp.44-49.
- [3]. Alhaithloul, H.A.S., Ali, B., Alghanem, S.M.S., Zulfiqar, F., Al-Robai, S.A., Ercisli, S., Yong, J.W.H., Moosa, A., Irfan, E., Ali, Q. and Irshad, M.A., 2023. “Effect of Green-Synthesized Copper Oxide NPS on Growth, Physiology, Nutrient Uptake, and Cadmium Accumulation in *Triticum Aestivum* (L.)” *Ecotoxicology and Environmental Safety*, 268, p.115701.
- [4]. Altuwirqi, R.M., Albakri, A.S., Al-Jawhari, H. and Ganash, E.A., 2020. “Green Synthesis of Copper Oxide NPS by Pulsed Laser Ablation in Spinach Leaves Extract”. *Optik*, 219,165280.
- [5]. Bradfield, S.J., Kumar, P., White, J.C. and Ebbs, S.D., 2017. “Zinc, Copper, or Cerium Accumulation from Metal Oxide Nanoparticles or Ions in Sweet Potato: Yield Effects and Projected Dietary Intake from Consumption.” *Plant Physiology and Biochemistry*, 110, pp.128-137.
- [6]. Da Costa, M.V.J. and Sharma, P.K., 2016. “Effect of Copper Oxide NPson Growth, Morphology, Photosynthesis, and Antioxidant Response in *Oryza Sativa*.” *Photosynthetica*, 54, pp.110-119.
- [7]. Di, X., Fu, Y., Huang, Q., Xu, Y., Zheng, S. and Sun, Y., 2023. “Comparative Effects of Copper NPS and Copper Oxide NPS on Physiological Characteristics and Mineral Element Accumulation in *Brassica Chinensis* L.” *Plant Physiology and Biochemistry*, 196, pp.974-981.
- [8]. Dwivedi, P., Kumar, S., & Vajpayee, P. (2024). “Copper Oxide Nanoparticles Influenced Ionome, Reduced Growth, Altered Pigments and Stomatal Morphology in *Mentha Arvensis*L.” *Environmental Geochemistry and Health*.
- [9]. Gaucin-Delgado, J.M., Ortiz-Campos, A., Hernandez-Montiel, L.G., Fortis-Hernandez, M., Reyes-Pérez, J.J., González-Fuentes, J.A. and Preciado-Rangel, P., 2022. “CuO-NPs Improve Biosynthesis of Bioactive Compounds in Lettuce.” *Plants*, 11(7), p.912.
- [10]. Gowtham, H.G., Shilpa, N., Singh, S.B., Aiyaz, M., Abhilash, M.R., Nataraj, K., Amruthesh, K.N., Ansari, M.A., Alomary, M.N. and Murali, M., 2024. “Toxicological Effects of NPS In Plants: Mechanisms Involved at Morphological, Physiological, Biochemical and Molecular Levels.” *Plant Physiology and Biochemistry*, 210, p.108604.
- [11]. Halfadji, A., Naous, M., Rajendrachari, S., Ceylan, Y., Ceylan, K.B. and Shekar, P.R., 2024. “Effective Investigation of Electro-Catalytic, Photocatalytic, and Antimicrobial Properties of Porous CuONPs Green Synthesized Using Leaves of *Cupressocyparis Leylandii*.” *Journal of Molecular Structure*, 1301, p.137318.
- [12]. Hasan, S., 2015. “A Review on Nanoparticles S: Their Synthesis and Types.” *Res. J. Recent Sci*, 2277, p.2502.
- [13]. Ji, H., Guo, Z., Wang, G., Wang, X. and Liu, H., 2022. “Effect of ZnO and CuONPson the Growth, Nutrient Absorption, and Potential Health Risk of the Seasonal Vegetable *Medicago Polymorpha*” *L. PeerJ*, 10, p.e14038.
- [14]. Joseph, T. M., Mahapatra, D. K., Esmaeili, A.,Piszczyk, Ł, Hasanin, M. S.,Kattali, M.,Haponiuk,J., Thomas,S., 2023. “Nanoparticles: Taking a Unique Position in Medicine”. *Nanomaterials (Basel)*.13(3):574. doi: 10.3390/nano13030574
- [15]. Kar, P., Muduli, S., Nayak, S., Nayak R. K., Parhi P., 2025. A Comprehensive Review on Biogenic Synthesis and Environmental - Applications of Precious Metal Nanoparticles”. *Journal of Environmental Chemical Engineering*13 (2025) 119164.
- [16]. Li, W., Tan, Y., Shang, G., Chen, L., Wu, Z., Lin, Y., Luo, L. and Yang, Y., 2024. “Analysis, Accumulation, Transformation, and Impact of Metallic NPsin Plants.” *Journal of Environmental Chemical Engineering*, p.114748.
- [17]. Liu, Z., Guo, S., Fang, X., Shao, X. and Zhao, Z., 2022. “Antibacterial and Plant Growth-Promoting Properties of Novel Fe₃O₄/Cu/CuO Magnetic NPs.” *RSC advances*, 12(31), pp.19856-19867.
- [18]. Margenot, A.J., Rippner, D.A., Dumlao, M.R., Nezami, S., Green, P.G., Parikh, S.J. and McElrone, A.J., 2018. “Copper Oxide Nanoparticle Effects on Root Growth and Hydraulic Conductivity of Two Vegetable Crops.” *Plant and Soil*, 431, pp.333-345.
- [19]. Pechyen, C., Tangnorawich, B., Toommee, S., Marks, R. and Parcharoen, Y., 2024. “Green Synthesis of Metal NPs, Characterization, and Biosensing

- Applications.” *Sensors International*, p.100287.
- [20]. Pelegriño, M.T., Kohatsu, M.Y., Seabra, A.B., Monteiro, L.R., Gomes, D.G., Oliveira, H.C., Rolim, W.R., De Jesus, T.A., Batista, B.L. and Lange, C.N., 2020. “Effects of Copper Oxide NPson Growth of Lettuce (*Lactuca Sativa L.*) Seedlings and Possible Implications of Nitric Oxide in Their Antioxidative Defence.” *Environmental Monitoring and Assessment*, 192, pp.1-14.
- [21]. Prakruthi, R. and Deepakumari, H.N., 2024. “CuONPs: Green Combustion Synthesis, Applications to Antioxidant, Photocatalytic and Sensor Studies.” *RSC advances*, 14(39), pp.28703-28715.
- [22]. Prerna, Dubey, A. and Ratan, G., 2021. “Nanoparticles: An Overview.” *Drugs and cell therapies in haematology*, 10(1), pp.1487-1497.
- [23]. Rajkumar, S., Elanthamilan, E., Wang, S.F., Chryso, H., Balan, P.V.D. and Merlin, J.P., 2022. “One-Pot Green Recovery of Copper Oxide NPS from Discarded Printed Circuit Boards for Electrode Material in Supercapacitor Application.” *Resources, Conservation and Recycling*, 180, p.106180.
- [24]. Rico, C.M., Majumdar, S., Duarte-Gardea, M., Peralta-Videa, J.R. and Gardea-Torresdey, J.L., 2011. “Interaction of Nanoparticles with Edible Plants and their Possible Implications in the Food Chain.” *Journal of agricultural and food chemistry*, 59(8), pp.3485-3498.
- [25]. Sharma, A., Sharma, R.B., Verma, A. and Thakur, R., 2022. “Insight on Nanoparticles, Green Synthesis and Applications in Drug Delivery System: A Comprehensive Review”. (2022). *Int. J. Life Sci. Pharma Res*, 12(5), pp.68-84.
- [26]. Singh, D. and Kumar, A., 2020. “Quantification of Metal Uptake in *Spinacia Oleracea* Irrigated with Water Containing a Mixture of CuOand ZnONPs.” *Chemosphere*, 243, p.125239.
- [27]. Singh, D., Sharma, A., Verma, S.K., Pandey, H. and Pandey, M., 2024. “Impact of NPS on Plant Physiology, Nutrition, and Toxicity: A Short Review.” *Next Nanotechnology*, 6, p.100081.
- [28]. Thandapani, G., Arthi, K., Pazhanisamy, P., John, J.J., Vinothini, C., Rekha, V., Santhanalakshmi, K. and Sekar, V., 2023. “Green Synthesis of Copper Oxide NPsUsing *Spinacia Oleracea* Leaf Extract and Evaluation of Biological Applications: Antioxidant, Antibacterial, Larvicidal and Biosafety Assay.” *Materials Today Communications*, 34, p.105248.
- [29]. Wang, W., Ren, Y., He, J., Zhang, L., Wang, X. and Cui, Z., 2020. “Impact of Copper Oxide NPson the Germination, Seedling Growth, and Physiological Responses in *Brassica Pekinensis L.*” *Environmental Science and Pollution Research*, 27, pp.31505-31515.
- [30]. Xiong, T., Dumat, C., Dappe, V., Vezin, H., Schreck, E., Shaid, M., Pierart, A., & Sobanska, S. (2017). “Copper Oxide Nanoparticle Foliar Uptake, Phytotoxicity, and Consequences for Sustainable Urban Agriculture.” *Environmental Science & Technology*, 51(7), 4012–4020.
- [31]. Xiong, T., Zhang, T., Xian, Y., Kang, Z., Zhang, S., Dumat, C., Shahid, M. and Li, S., 2021. “Foliar Uptake, Biotransformation, and Impact of CuONPsin *Lactuca Sativa L. Var. RamosaHort*”. *Environmental Geochemistry and Health*, 43, pp.423-439.