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Department of Medical Physics

Effect PH on Capacity of Cuo For Antibacterial Activity

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

(هُوَ الَّذِي جَعَلَ الشَّمْسَ ضِيَاءً وَالْقَمَرَ نُورًا وَقَدَّرَهُ مَنَازِلَ لِتَعْلَمُوا عَدَدَ السِّنِينَ وَالْحِسَابَ مَا خَلَقَ اللَّهُ ذَلِكَ إِلَّا بِالْحَقِّ يُفَصِّلُ الْآيَاتِ لِقَوْمٍ يَعْلَمُونَ). سورة يونس- الآية 5.

صدق الله العظيم

الاهداء

من قال أنا لها.... نالها

وأنا لها وإن أبت رغما عنها أتيت بها

لم تكن الرحلة قصيرة ولا ينبغي لها أن تكون ولم يكن الحلم قريبا ولا الطريق كان محفوفا بالتسيهلات لكنني فعلتها ونتلها

الى من شرفني بحمل اسمه.. والدي العزيز ...الى النور الذي أنار دربي والسراج الذي لاينطفي نوره بقلبي أبدا من بذل الغالي والنفيس واستمديت منه قوتي واعتزازي بذاتي..

إلى نور عيني وضوء دربي ومهجة حياتي إلى التي ساندتني ووقفت بجانبي وقدمت لي الدعم لمواصلة طريقي إلى التي وهبتني الحياة والأمل واحتضنني قلبها قبل يدها وسهلت لي الشدائد بدعائها والدتي الحبيبة ...

الى أستاذتي الكرام ممن لم يتوانوا في مد يد العون لناا... يا من صنعتم لنا المجد أهديكم هذا الانجاز وثمره نجاحي الذي لطالما تمنيتها أنا اليوم أتممت أول ثمراته بفضل من الله عز وجل فالحمدلله على ماوهبني وأن يعينني ويجعلني مباركة أينما كنت .

الشكر و التقدير

الى اساتذتي الافاضل ... الى رسل العلم

و اتوجه بالشكر الجزيل الى

م.م. رغد احمد

التي تفضلت بالأشراف على هذا البحث فجزاها الله كل خير

فلها كل التقدير و الاحترام

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Abstract

Well-structured nano-sized Copper oxide has been prepared by simple reflex method for different pH concentrations. The Prepared samples are subjected to various characterization studies such as XRD, SEM, UV–Vis spectroscopy and FTIR spectroscopy in order to investigate their crystallite structure, morphology, optical properties and functional vibrations. The structural analysis of prepared CuO samples revealed a monoclinic crystalline structure. The morphology of CuO samples reveals spindle shaped with size distribution ranging from 70 nm to 90 nm. The sample prepared at pH 5 seemed to possess the expected qualities to apply for antibacterial activity. The prepared samples consisting of various amounts of CuO nanoparticles are developed to study the antibacterial activity for different strains of bacteria. The results showed that at the optimized pH concentration, CuO samples calcinated at 400 °C exhibited improved antibacterial activity for E. coli bacteria.

1- Introduction

Fabrication of transition metal oxides at nano regime has received a greater attention among material science researchers due to its exceptional properties Among the existing transitional metal oxide copper oxide attracted considerable research in recent days. Copper oxide has two forms with respect to its valences such as, cuprous oxide (Cu_2O) and cupric oxide (CuO). Because of their unique properties such as their environmental friendliness, natural abundance and high optical absorption coefficients, the two oxides have emphasized in various applications like pseudo capacitor electrode, magnetic phase transition, battery application, biocidal activity, antibacterial inhibition analysis, waste water treatment and purification etc. Even though copper complexes exhibit insignificant sensitivity on human tissue, they show good inhibition against bacterial growth. Recent research picturizes that nanoparticle such as silver, copper, gold, zinc and their oxides maximize the therapeutic effects. Moreover. Copper oxide nanoparticles has drawn a great attention due to its efficient bactericidal activity, adhesion performance. Also, copper oxide complexes at nano range can be a good candidate because of its consumable price. [1]

1.1 Definition of Potential of Hydrogen (PH)

PH, quantitative measure of the acidity or basicity of aqueous or other liquid solutions. The term, widely used in chemistry, biology, and agronomy, translates the values of the concentration of the hydrogen ion—which ordinarily ranges between about 1 and 10^{-14} gram-equivalents per litre—into numbers between 0 and 14. In pure water, which is neutral (neither acidic nor alkaline), the concentration of the hydrogen ion is 10^{-7} gram-equivalents per litre, which corresponds to a pH of 7. A solution with a pH less than 7 is considered acidic; a solution with a pH greater than 7 is considered basic, or alkaline.[2]

The measurement was originally used by the Danish biochemist S.P.L. Sørensen to represent the hydrogen ion concentration, expressed in equivalents per litre, of an aqueous solution: $pH = -\log[H^+]$ (in expressions of this kind, enclosure of a chemical symbol within square brackets denotes that the concentration of the symbolized species is the quantity being considered).[3]

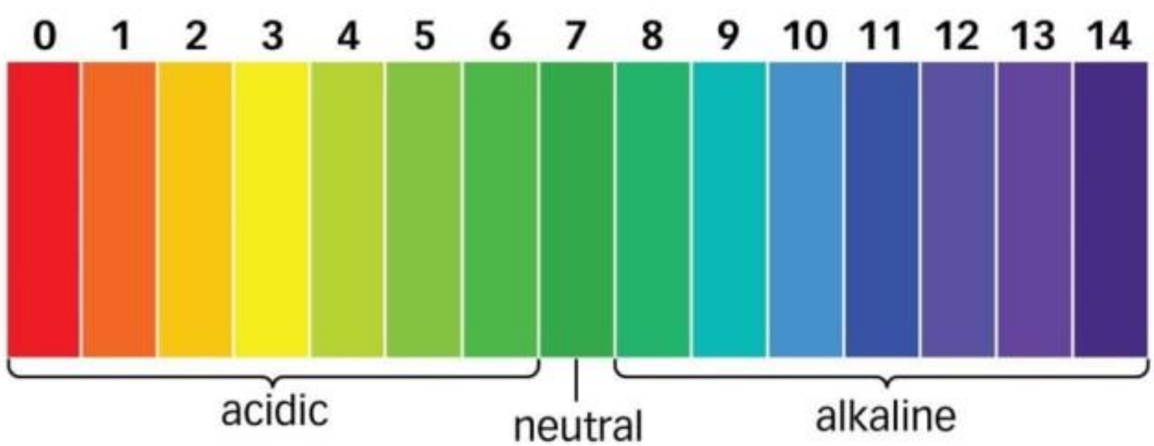


Fig (1-1):- potential of hydrogen

1.2 Methods of Synthesis for Biomedical CuO Nanoparticles

The synthesis approaches of CuO Nanoparticles (NPs) have advanced significantly in the last ten years because of their important biomedical and industrial applications [4]. The synthesis technique is important for the properties of the final nanosystem, since it may control the size and morphology of the nanoparticles. Also, these nanoparticles present various optical and magnetic properties, mechanical strengths and electrical resistivity, which differ from the characteristics of bulk solid material. Several methods for the synthesis of CuO NPs have been used, and the most relevant approaches, along with the typical resulting particle sizes, are listed in Table 1.

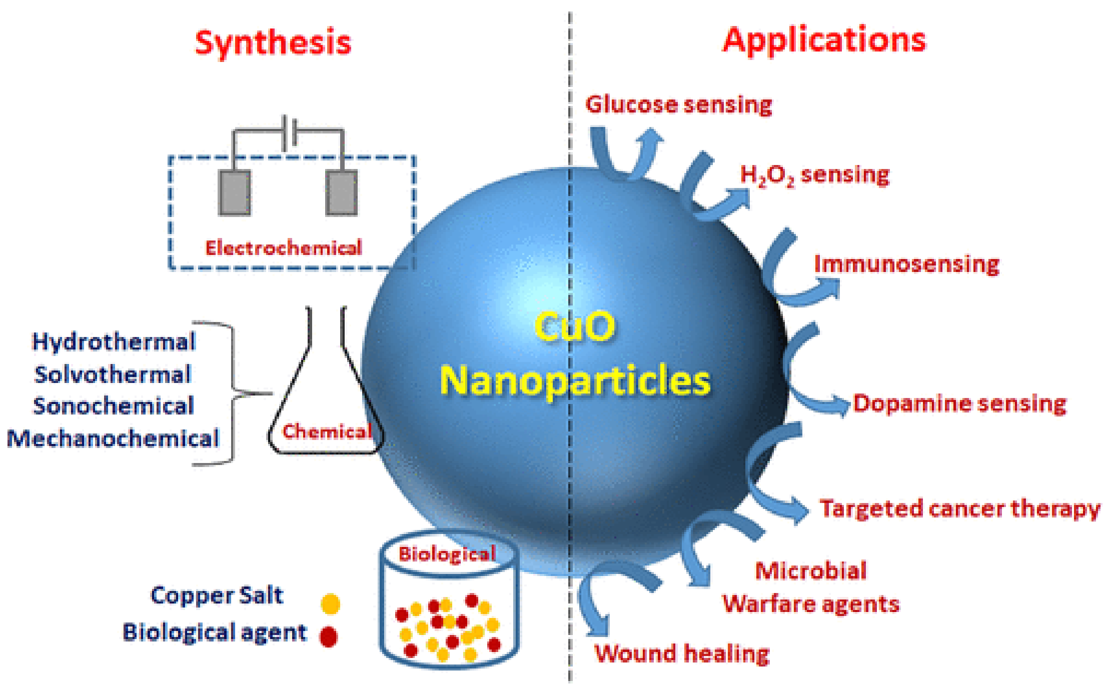


Fig (1-2):- Synthesis and Biomedical Applications of Copper Oxide Nanoparticles

Table 1:- The synthesis of CuO NPs with different methods results in different sizes [5].

Preparation Method	Size (nm)
Electrochemical method	4
Sonochemical synthesis	20–30
Sol-gel techniques	7–9
Microemulsion system	5–25

1.2.1 Electrochemical Method

The electrochemical method was invented by Switzer as a way to synthesize ceramic films. Since then this method has been continuously used for the preparation of nano-metal oxides such as ZnO, CuO, etc. The first reported CuO nanocrystals were prepared by using Cu as a sacrificial anode [6].

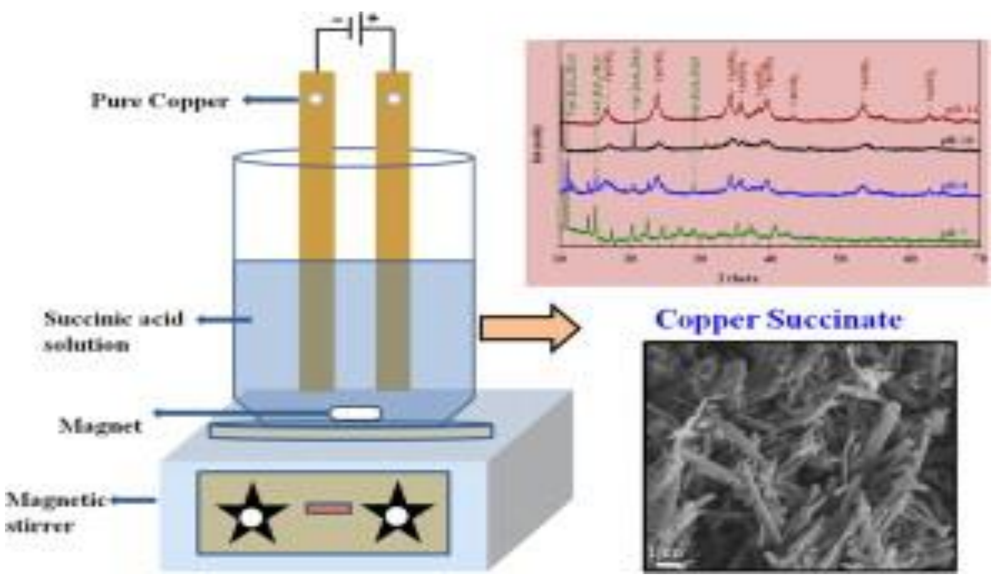


Fig (1-2):- Copper succinate nanoparticles synthesis by electrochemical method

1.2.2 Sonochemical Method

The sonochemical method is a simple process that follows three steps: (1) formation, (2) development, (3) the implosive collapse of the obtained microcavities. The method involves the application of ultrasound during the synthesis of the product [7].

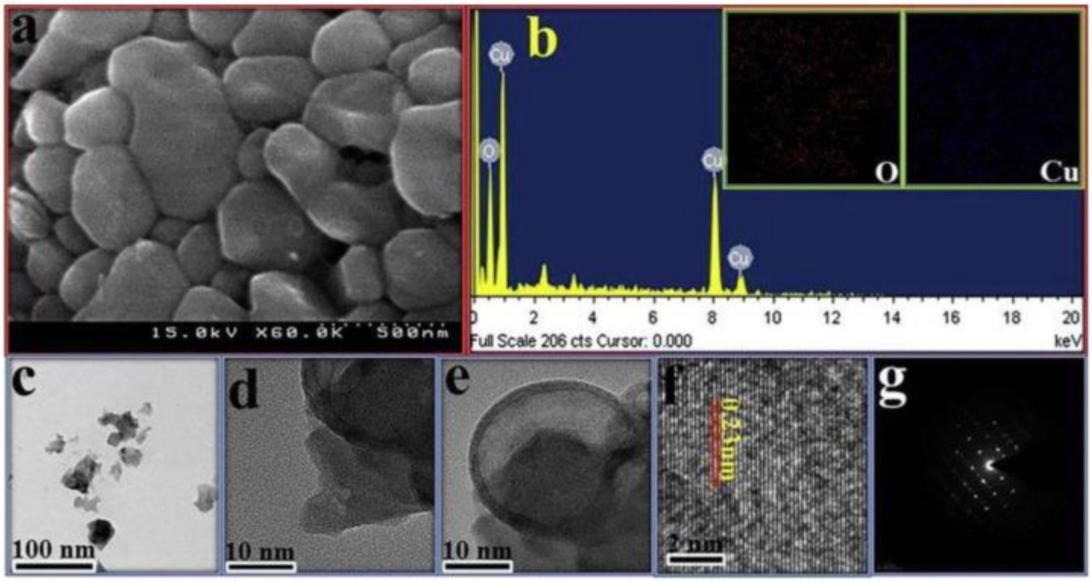
1.2.3 Sol-Gel Method

The sol-gel technique is a simple and relatively fast method and therefore it is widely used in the design of nanoparticles [8]. This method is applied often as it ensures the rigorous control of the nanoparticle size.

1.2.4 Other Synthetic Methods

Other methods for the synthesis of CuO NPs have been developed; such as hydrothermal approach, thermal oxidation method, alcohol-thermal synthesis, liquid ammonia and microwave-assisted synthesis [9].

Direct thermal decomposition method is broadly used for the synthesis of CuO NPs. One approach consists in adding sodium carbonate to copper sulfate and by calcination spherical CuO NPs are formed [10].



Fig(1.2) :- Microscopic images of CuO NPs synthesized by a green method: (**a**) scanning electron microscopy (SEM) image; (**b**) EDAX spectrometry of the CuO NPs; inset: elemental mapping of oxygen and copper; (**c**–**e**) transmission electron microscopy (TEM) images at different magnifications; (**f**) high magnification view of the CuO NPs; and (**g**) Selected Area Electron Diffraction (SAED) pattern of the CuO NPs [11].

1.3 Medical Applications

CuO nanoparticles may have different applications depending on the various properties they manifest, which are highly influenced by their size, surface properties, optical and magnetic traits, the synthesis method being an important parameter for controlling all these and thus, their biological properties. Some of these applications include doping materials in semiconductors, such as chemical sensors, antimicrobial agents, catalyst for different cross coupling reactions, anti-cancer formulations, coating materials etc. Future biomedical applications of CuO NPs are focused intensively on disease detection and could present potential applications in many other areas, for example, in the detection of viruses in the human body [12]. In a recent study, Li et al. developed a highly sensitive and selective method for the detection of H1N1 flu virus. The principle of this method is based on labeling of antibodies by using CuO NPs. This approach was designed a sandwich complex made of CuO NPs labeled polyclonal antibody, able to detect and bind antigens represented by the H1N1 virus [13]. The method is an enzymatic chromogenic approach, belonging to the so-called enzyme linked immunosorbent assay (ELISA) methods, and proved to be highly sensitive and faster, as compared with other related methods.

1.3.1 Antibacterial Activity of CuO

Although the specific mechanism of the antimicrobial effect related with the use of CuO nanoparticles is not known, several of their mechanisms of action on bacterial cells have been discussed. Even if not specific to CuO nanoparticles, but for most oxide nanoparticles, Zhang et al. reported that the generation of reactive oxygen species (ROS) within bacterial cells is enhanced when using CuO-water suspensions.[7]

The antibacterial activity of CuO NPs seems to be different depending on the particularities of bacteria cells. For examples, their cellular walls seem to impact the antimicrobial effect of CuO NPs, Gram character being a key aspect. It was reported that 100% of *E. coli* cells, which are Gram negative, were killed when a concentrations of CuO NPs higher than 9.5% was used, while for the Gram positive species *Staphylococcus aureus* the killing ability was lower [14]. It was also reported that CuO nanoparticles inhibit the growth of *E. coli*, *Pseudomonas aeruginosa*, and *S. aureus* in a time dependent manner, the utilized dose being, of course, the most important factor [15].

Goyal et al. also reported that the antimicrobial properties depend on the surface properties and size of nanoparticles. It seems that small particles with a large surface area have better antibacterial activity, as compared with larger ones. CuO NPs showed a major antimicrobial activity also against *Bacillus subtilis* [16]. El-Nahhal et al. tested the antibacterial activity of CuO NP-coated cotton dressings and CuS nanoparticle-coated cotton dressings. Both were inoculated with *E. coli* and *S. aureus* in order to compare the antimicrobial effect of the two coating systems in a

Gram negative and Gram positive model, respectively. The results showed that the sample with CuO NPs presented higher antibacterial activity than the sample coated with CuS nanoparticles which showed no reduction in the viability of tested bacteria [17]. Devi et al. studied the antimicrobial activity of bulk, as-prepared and annealed CuO NPs against *E. coli*, *Proteus mirabilis*, *Klebsiella* spp., and their effect was comparable with the antimicrobial activity of gentamycin on these strains [4,18].

The antibacterial effect of copper NPs was analyzed on the basis of the zone of inhibition. CuO NPs exhibited antibacterial activity against both gram-positive and gram-negative bacteria such as *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa* by the disc diffusion method. The synthesized CuO NPs show effects against gram positive and gram negative bacteria, respectively, with a zone of inhibition of 12 mm and 10 mm in Table 1. These results were also compared with those of standard antibiotics.[19]

Obtained experimental results have shown that synthesized CuO NPs have antibacterial activity on both gram-positive and gram-negative bacteria.

Table 2: Antibacterial activity of CuO NPs using some human pathogenic bacteria by disc diffusion method

Test organism	**Zone of inhibition (mm)**
Staphylococcus aureus	12
Escherichia coli	10
CuO NPs: Copper oxide nanoparticles	

1.3.2 Effect the pH on the capacity of CuO

The pH values of the precursor, the extract, and the NPs before pH was adjusted were 4, 5, and 5.4, respectively. The CuO NPs synthesized at different pH values (6, 7, 8, 9.5, 10, 11, and 12) were optimized based on the intensity of their peak and the red shift of their respective λmax values. The best result for the synthesis of CuO NPs was obtained at pH=11. This shows that a more basic media is very suitable for the synthesis of CuO NPs. [20]

1.4 Previous studies

1.4.1 Hsueh, Yi-Huang, Ping-Han Tsai, and Kuen-Song Lin. "pH-dependent antimicrobial properties of copper oxide nanoparticles in Staphylococcus aureus." ***International journal of molecular sciences*** **18.4 (2017): 793.**

The antimicrobial properties of CuO nanoparticles have been investigated, but the underlying mechanisms of toxicity remain the subject of debate. Here, we show that CuO nanoparticles exhibit significant toxicity at pH 5 against four different Staphylococcus aureus (S. aureus) strains, including Newman, SA113, USA300, and ATCC6538. At this pH, but not at pH 6 and 7, 5 mM CuO nanoparticles effectively caused reduction of SA113 and Newman cells and caused at least 2 log reduction, whereas 20 mM killed most strains but not USA300. At 5 mM, the nanoparticles were also found to dramatically decrease reductase activity in SA113, Newman, and ATCC6538 cells, but not USA300 cells. In addition, analysis of X-ray absorption near-edge structure and extended X-ray absorption fine structure confirmed that S. aureus cells exposed to CuO nanoparticles contain CuO, indicating that Cu2+ ions released from nanoparticles penetrate bacterial cells and are subsequently oxidized intracellularly to CuO at mildly acidic pH. The CuO nanoparticles were more soluble at pH 5 than at pH 6 and 7. Taken together, the data conclusively show that the toxicity of CuO nanoparticles in mildly acidic pH is caused by Cu2+ release, and that USA300 is more resistant to CuO nanoparticles (NPs) than the other three strains.

1.4.2 Taran, Mojtaba, Maryam Rad, and Mehran Alavi. "Antibacterial activity of copper oxide (CuO) nanoparticles biosynthesized by Bacillus sp. FU4: optimization of experiment design." ***Pharmaceutical Sciences*** **23.3 (2017): 198-206.**

Background: There are several methods for synthesis of metallic nanoparticles (NPs) including chemical, physical and biological process. In this study, Bacillus sp. FU4 was used as biological source for biosynthesis of CuO NPs. Methods: CuO NPs have been prepared by copper sulfate (CuSO4). CuO NPs were formed after oxidation of Cu NPs. Design and analysis of Taguchi experiments (an orthogonal assay and analysis of variance (ANOVA)) carried out by the Qualitek-4 software. Average effect of CuSO4 concentration (0.1, 0.01 and 0.001 M), incubation and culturing time (48, 72, 96 hours) as three controllable factors with three levels were evaluated in CuO NPs biosynthesis. Characterization of CuO NPs was determined by UV-Vis spectroscopy, X-ray diffraction (XRD), Fourier transform infra-red (FT-IR) spectroscopy and scanning electron microscopy (SEM). Also, the antimicrobial properties of CuO NPs were investigated using Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 43300 as multidrug resistant (MDR) bacteria. Results: Results: It was evaluated that, NPs size distributions were in the range of 2-41 nm with spherical shapes. The anti-bacterial activities of CuO NPs were measured based on diameter of inhibition zone in disk diffusion tests of NPs dispersed in batch cultures. Two levels of CuSO4 concentrations (0.1 and 0.01M) had antibacterial effect on E.coli (33±0.57 and 6 ±2mm). In the case of S. aureus, there was surprisingly no sign of growth. Conclusion: CuO NPs have antibacterial activity that can be benefit in medicinal aspect for fighting against prominent pathogen bacteria such as E.coli ATCC 25922 and S.aureus ATCC 43300.

1.4.3 George, A., Raj, D. M. A., Raj, A. D., Irudayaraj, A. A., Arumugam, J., Prabu, H. J., ... & Kaviyarasu, K.,Temperature effect on CuO nanoparticles: antimicrobial activity towards bacterial strains, (2020),*Surfaces and Interfaces*, *21*, 100761.

Semiconductor metal oxides are under investigation in recent years towards biological applications due to their high stability. Especially CuO nanoparticles have gained attention due to their low cost and easy synthesis. Herein, the effect of calcinations temperature on the CuO nanospindles prepared by reflux method were investigated. The XRD results reveal a monoclinic structured CuO formation. The morphology analyzed with SEM projected a decrease in particle size at higher calcinations temperature of 800 °C. The optical bandgap calculated from *Tauc plot* also showed an increase in bandgap value at higher calcinations temperature. The antibacterial property of the sample with more surface to volume ratio has been studied towards a gram-positive and a gram-negative bacterial strain. The prepared CuO nanoparticles showed better reactivity towards the gram-negative *E.coli* when compared to that of gram-positive staphylococcus aureus. Moreover, the antibacterial results reveal that the sensitivity towards bacterial strains is proportional to the concentration of the CuO sample were reported in detail.

1.4.4 Basay Jr, C. P., Delmo-Organo, N., Villegas, L. C., Fernando, L. M., Pide, J. L. V., Madayag, R. E., ... & Paterno, E. S. ,Effect of ZnO and CuO Nanoparticles on Culturable Bacterial Population, Microbial Biomass, and Enzyme Activities in Two Soil Types. *Philippine Journal of Science*, (2022), *151*(6A), 2159-2172.

Metal oxide nanoparticles are widely used in many agricultural, medical, and electronic products. This study was conducted to assess the effects of metal oxide nanoparticles on the total culturable soil bacterial population and enzymatic activities – namely, dehydrogenase (DHA), urease (UA), and microbial biomass (MCB) – using Philippine soils. The factors included the two soil types – Lipa clay loam (LCL) and Sariaya sandy loam (SSL) – and five treatments – namely, copper oxide bulk particles (bCuO), zinc oxide bulk particles (bZnO), copper oxide nanoparticles (CuONPs), zinc oxide nanoparticles (ZnONPs), and untreated control. The experiment was laid out in a split-plot completely randomized design over a 28-day incubation period under laboratory conditions. Results showed no significant effect on the culturable bacterial population. The DHA of ZnONPs-, bCuO-, and CuONPs-treated LCL was significantly reduced up to 7, 14, and 28 days after amendment (DAA), respectively. This result was probably due to the strongly acidic property, high level of organic matter (OM), low permeability due to particle size distribution, and low phosphate concentration of LCL. For UA, it was significantly reduced in LCL by CuONPs up to 28 DAA, perhaps due to the inactivation of soil UA. UA, however, was significantly increased by ZnONPs up to 28 DAA, presumably due to the release of intracellular urease from dead cells due to the toxic level of Zn2+. Soil MCB was significantly reduced at 7 DAA in CuONP-amended LCL and ZnONPsamended SSL, whereas bCuO- and bZnO-amended LCL had a significant increase in MCB. The effect of CuONPs and ZnONPs on the parameters measured was influenced by soil type. In conclusion, CuONPs could pose a threat to soil microorganisms, especially in clayey soils.

1.4.5 Ślosarczyk, Agnieszka, et al. "Antimicrobial action and chemical and physical properties of CuO-doped engineered cementitious composites." *Scientific Reports* 13.1 (2023): 10404.

CuO nanoparticles (NPs) were added to cement matrices in quantities of 0.25, 0.50 and 1.00 wt% to inhibit the growth of Gram-positive (*Bacillus* *cereus*, *Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*) bacteria. It was shown that CuO NPs, in all tested concentrations, improved the antibacterial properties of the cement matrix. Nevertheless, the best mechanical, structural and durability properties were obtained for cement composites doped with CuO NPs at 0.25 wt%. Larger amounts of NPs caused a decrease in all parameters relative to the reference mortar, which may be the result of a slight change in the porosity of the composite microstructure. For 0.50 wt% CuO NPs, a slight increase in the volume of micropores in the cement matrix was observed, and an increased number of larger pores was confirmed by non-invasive computed tomography (CT). The reduction in the mechanical parameters of composites with 0.50 and 1.00 wt% CuO NPs may also be due to the slower hydration of the cement binder, as confirmed by changes in the heat of hydration for these configurations, or agglomeration of NPs, especially for the 1.00 wt% concentration, which was manifested in a decrease in the plasticity of the mortars.

Conclusion

The conclusion regarding the effect of pH on the capacity of CuO (copper oxide) for antibacterial activity would depend on the specific experimental findings and data analysis conducted in a study. However, generally speaking, here are some potential conclusions that could be drawn Based on previous studies:

1. Optimal pH for Antibacterial Activity: If the study finds that CuO exhibits the highest antibacterial activity at a particular pH range, the conclusion would be that pH significantly influences CuO's capacity for antibacterial activity. This could suggest that controlling the pH of the environment where CuO is used may enhance its antibacterial efficacy.
2. pH Dependency of Antibacterial Activity: If the antibacterial activity of CuO varies significantly across different pH levels, the conclusion would be that pH exerts a pronounced effect on CuO's antibacterial capacity. This implies that pH needs to be considered as a critical factor when utilizing CuO for antibacterial purposes.
3. pH-Insensitive Antibacterial Activity: Conversely, if the study finds that changes in pH have minimal to no effect on CuO's antibacterial activity, the conclusion would be that pH does not significantly influence CuO's capacity for antibacterial activity. This suggests that other factors may play a more dominant role in determining CuO's antibacterial efficacy.
4. Complex Relationship with pH: In some cases, the relationship between pH and CuO's antibacterial activity may not be straightforward, showing a complex pattern. The conclusion would involve acknowledging this complexity and potentially exploring further research to elucidate the underlying mechanisms governing this relationship.
5. Potential Practical Implications: Regardless of the specific findings, the conclusion would likely discuss the practical implications of the relationship

between pH and CuO's antibacterial activity. This could include recommendations for optimizing conditions for antibacterial applications or suggestions for further research to better understand the underlying mechanisms.

Overall, the conclusion would summarize the key findings regarding the effect of pH on CuO's antibacterial capacity, discuss the implications of these findings, and potentially suggest avenues for future research or practical applications.

الخاتمة

الحمد لله تعالى الذي وفقنا في تقديم هذا المشروع ، وها هي القطرات الأخيرة في مشوار هذا المشروع ، وقد بذلنا كل الجهد والبذل لكي يخرج هذا المشروع في هذا الشكل. ونرجو من الله أن تكون رحلة ممتعة وشيقة ، وكذلك نرجو أن تكون قد ارتقت بدرجات العقل الفكر ، حيث لم يكن هذا الجهد بالجهد اليسير ، ونحن لا ندعى الكمال فإن الكمال لله عز وجل فقط ، ونحن قد قدمنا كل الجهد لهذا المشروع ، فإن وفقنا فمن الله عز وجل وإن أخفقنا فمن أنفسنا ، وكفانا نحن شرف المحاولة .

واخيراً نرجو أن يكون هذا المشروع قد نال إعجابكم . وصل اللهم وسلم وبارك تسليما كثيراً على معلمنا الأول وحبيبنا سيدنا محمد عليه أفضل الصلاة والسلام .

References

[1]- Raj, D. M. A., George, A., Raj, A. D., Irudayaraj, A. A., Venci, X., Arumugam, J., ... & Prabu, H. J. (2021). The role of pH in enhancing the capacity of CuO nanoparticles for antibacterial activity. *Materials Today: Proceedings*, *36*, 504-508.

[2]- Horne, A. J., and Goldman, C. R. 1994. Limnology, 2nd edition. McGraw-Hill, Inc. 576 pp.

[3]- https://www.britannica.com/science/pH

[4]- Devi A.B., Moirangthem D.S., Talukdar N.C., Devi M.D., Singh N.R., Luwang M.N. Novel synthesis and characterization of CuO nanomaterials: Biological applications. *Chin. Chem. Lett.* 2014;25:1615–1619. doi: 10.1016/j.cclet.2014.07.014.

[5]- Dagher S., Haik Y., Ayesh A.I., Tit N. Synthesis and optical properties of colloidal CuO nanoparticles. *J. Lumin.* 2014;151:149–154. doi: 10.1016/j.jlumin.2014.02.015.

[6]- Yuan G.-Q., Jiang H.-F., Lin C., Liao S.-J. Shape-and size-controlled electrochemical synthesis of cupric oxide nanocrystals. *J. Cryst. Growth.* 2007;303:400–406. doi: 10.1016/j.jcrysgro.2006.12.047.

[7]- Zhang Q., Zhang K., Xu D., Yang G., Huang H., Nie F., Liu C., Yang S. CuO nanostructures: Synthesis, characterization, growth mechanisms, fundamental properties, and applications. *Prog. Mater. Sci.* 2014;60:208–337. doi: 10.1016/j.pmatsci.2013.09.003.

[8]- Jayaprakash J., Srinivasan N., Chandrasekaran P. Surface modifications of CuO nanoparticles using Ethylene diamine tetra acetic acid as a capping agent by sol–gel routine. *Spectrochim. Acta A. Mol. Biomol. Spectrosc.* 2014;123:363–368. doi: 10.1016/j.saa.2013.12.080

[9]- Karunakaran C., Manikandan G., Gomathisankar P. Microwave, sonochemical and combustion synthesized CuO nanostructures and their electrical and bactericidal properties. *J. Alloys Compd.* 2013;580:570–577. doi: 10.1016/j.jallcom.2013.07.150.

[10]- Suleiman M., Mousa M., Hussein A., Hammouti B., Hadda T.B., Warad I. Copper (II)-oxide nanostructures: Synthesis, characterizations and their applications–review. *J. Mater. Environ. Sci.* 2013;4:792–797.

[11]- Nagajyothi P.C., Muthuramanb P., Sreekanthc T.V.M., Kimb D.H., Shim J. Green synthesis: In-vitro anticancer activity of copper oxide nanoparticles against human cervical carcinoma cells. *Arab. J. Chem.* 2016 doi: 10.1016/j.arabjc.2016.01.011.

[12]- Ahamed M., Alhadlaq H.A., Khan M., Karuppiah P., Al-Dhabi N.A. Synthesis, characterization, and antimicrobial activity of copper oxide nanoparticles. *J. Nanomater.* 2014; 5:519–524. doi: 10.1155/2014/637858.

[13]- Li Y., Hong M., Lin Y., Bin Q., Lin Z., Cai Z., Chen G. Highly sensitive electrochemical immunoassay for H1N1 influenza virus basedon copper-mediated amplification. *Chem. Commun.* 2012; 48:6562–6564. doi: 10.1039/c2cc31990j.

[14]- Ungur G., Hrůza J. Influence of copper oxide on the formation of polyurethane nanofibers via electrospinning. *Fibers Polym.* 2015;16:621–628. doi: 10.1007/s12221-015-0621-9.

[15]- Das D., Nath B.C., Phukon P., Dolui S.K. Synthesis and evaluation ofantioxidant and antibacterial behavior of CuO nanoparticles. *Colloids Surf. B Biointerfaces.* 2013;101:430–433. doi: 10.1016/j.colsurfb.2012.07.002.

[16]- Goyal R., Macri L.K., Kaplan H.M., Kohn J. Nanoparticles and nanofibers for topical drug delivery. *J. Control. Release.* 2015 doi: 10.1016/j.jconrel.2015.10.049.

[17]- El-Nahhal I.M., Zourab S.M., Kodeh F.S., Selmane M., Genois I., Babonneau F. Nanostructured copper oxide-cotton fibers: Synthesis,

characterization, and applications. *Int. Nano Lett.* 2012;2:1–5. doi: 10.1186/2228-5326-2-14.

[18]- Naika H.R., Lingaraju K., Manjunath K., Kumar D., Nagaraju G., Suresh D., Nagabhushana H. Green synthesis of CuO nanoparticles using Gloriosa superba L. extract and their antibacterial activity. *J. Taibah Univ. Sci.* 2015;9:7–12. doi: 10.1016/j.jtusci.2014.04.006.

[19]- SALOKI, A., & DAHARWAL, S. J. (2023). GREEN SYNTHESIS OF COPPER OXIDE NANOPARTICLE FROM PLANT EXTRACT AND ITS ANTIBACTERIAL ACTIVITY. *Asian J Pharm Clin Res*, *16*(7), 172-176.

[20]- Chauhan RP, Gupta C, Prakash D. Methodological advancements in green nanotechnology and their applications in biological synthesis of herbal nanoparticles. Int J Bioassays 2012;1:6-10.1