INTERDISIPLIN

Journal of Qualitative and Quantitative Research

ISSN: 3031-4828 Vol. 2 No. 5 (2025)

DOI: https://doi.org/10.61166/interdisiplin.v2i5.125 pp. 347-356

Research Article

Investigation of Antibacterial Activity of Biosynthetic Copper Oxide Nanoparticles

Azizi Karam Khan[1], Usefzay Obaidullah[2], Anwari Gulaqa[3]

1. Department of Biology, Faculty of Education, Panjshir Higher Education Institute, Panjshir 1501, Afghanistan; Azizikaramkhan11@gmail.com
2. Department of Biology, Faculty of Education, Alberoni University, Kapisa 1254, Afghanistan. E-mail: usefzay@gmail.com
3. Department of Agronomy, Faculty of Agriculture, Kunduz University Kunduz 3501, Afghanistan. Email: gulaqa.anwari@gmail.com

Copyright © 2025 by Authors, Published by **INTERDISIPLIN: Journal of Qualitative and Quantitative Research.** This is an open access article under the CC BY License https://creativecommons.org/licenses/by/4.0/

Received : July 25, 2025 Revised : August 13, 2025

Accepted : September 11, 2025 Available online : October 19, 2025

How to Cite: Karam Khan Azizi, Usefzay Obaidullah, & Gulaqa Anwari. (2025). Investigation of Antibacterial Activity of Biosynthetic Copper Oxide Nanoparticles. *INTERDISIPLIN: Journal of Qualitative and Quantitative Research*, 2(5), 347–356. https://doi.org/10.61166/interdisiplin.v2i5.125

Abstract. The present study aims to investigate the antibacterial activity of copper oxide nanoparticles synthesized by the gram-negative bacterium Escherichia coli. Biosynthetic CuONPs produced were tested on two pathogenic model bacteria, such as Pseudomonas aeruginosa (PTTC 1707) and Bacillus cereus (PTTC1154). The antibacterial effect of the mentioned nanoparticles was investigated using two methods of determining (MIC and well diffusion). Dilution series were used to determine the zone of no growth in the well diffusion method from 160 to 5 µg/mL, and the diameters of the formed zones were measured in milliliters. Also, to determine the minimum growth inhibitory concentration (MIC) of each bacterium, a series of 10 tubes with dilutions of copper oxide nanoparticles from 320 to 0.62

μg/mL was used. The results of the findings indicated that these nanoparticles showed significant antibacterial activity on the tested strains, such that they prevented the growth of bacteria at very low concentrations. The minimum inhibitory concentration (MIC) of copper oxide nanoparticles for both bacteria (Bacillus cereus) and (Pseudomonas aeruginosa) was calculated to be 10 and 5 micrograms ml, respectively. The diameters of the halos formed by these two bacteria were also calculated to be 17 and 20 mm, respectively.

Keywords: Biosynthesis, antibacterial properties, copper oxide nanoparticles, Escherichia coli.

INTRODUCTION

Copper, with the symbol (Cu), is a chemical element with atomic number 29, and is located in group 11 of the periodic table of elements. The good characteristics of copper metal include its ductility, malleability, thermal conductivity, and excellent electrical conductivity *(William, et al., 2011)*. Copper metal has been used as a disinfectant compound among nanoparticles throughout the history of human civilization due to its antibacterial and antiviral properties *(Borkow, et al., 2005)*. Oxide nanoparticles, often referred to as ceramic oxide nanoparticles, include a wide range of nanoparticles that constitute the most widely used category of nanomaterials. Among these nanoparticles are copper oxide nanoparticles. Due to the quantum measure effect and the vast, adequate level region, these NPs exhibit unique physical and chemical properties that play a significant role in today's technological and industrial world *(Sharma, et al., 2015)*. Copper oxide is a semiconductor compound that exhibits unique properties, including high electrical conductivity, curative resistance, and corrosion resistance. Copper oxide nanoparticles have many commercial applications among transition metal oxides. These applications include biocidal activity, magnetic phase transfer, drug delivery, biosensors, and catalytic properties *(Thakkar, et al., 2010; Mandal, et al., 2006)*.

The use of metals as disinfectants and antibacterial agents has been popular for a long time. Still, with the advent of modern and combined antibiotics that were more effective and faster, and also due to the destructive side effects of metals and their compounds on living tissues, this feature was forgotten for a long time *(Hajipour, et al., 2012)*.

In recent years, the amazing advances in nanoscience and the subsequent discovery of new properties of materials at the nanoscale have led researchers to seek the key to the problem of the increasing resistance of pathogenic microorganisms to metal nanoparticles. In addition to having amazing properties and high potential in biomedical applications, metal nanoparticles lack the destructive effects of metals and their ions in bulk on human health *(Gold, et al., 2018; Lewinski, et al., 2008)*.

So far, numerous nanostructures have become checked to the antibacterial with antifungal activities; when examining these nanostructures, it should always be kept in mind that some metals (such as copper, zinc, and silver) exhibit antimicrobial properties even in the bulk state, while others (such as iron oxide) lack antimicrobial properties in the bulk state but may exhibit antibacterial and antifungal properties in the nano scale *(Thit, et al., 2015)*. When nanoparticles are placed in biological culture media, they encounter different biological surfaces. These biological surfaces are due to the presence of various compounds intracellularly and extracellularly, including DNA, proteins, lipids, polysaccharides, flavonoids, etc., each of which has its unique characteristics. Therefore, the fate and function of a nanoparticle within a biological system depend on its numerous physicochemical properties and, as a result, the interactions created with biological surfaces. Copper oxide nanoparticles, due to their tiny dimensions, have a high surface-to-volume ratio, and this characteristic leads to the concentration of a large amount of free energy on their surface. In order to reduce this energy and achieve greater stability, and due to the electrostatic forces present in biological environments, nanoparticles collide with various biological surfaces. Those interactions that can overcome the energy barrier of the nanoparticle at an appropriate distance cause the creation of an electron-hole pair with the same free electron. Free electrons within biological systems lead to the production of reactive oxygen species, which subsequently induce oxidative stress *(Gold, et al., 2018; Zhao, et al., 2011)*.

As a general rule, small-sized nanoparticles (generally less than 30 nm) often lead to bacterial death by penetrating the cell membrane and interfering with membrane processes. Next, larger nanoparticles are absorbed based on electrostatic interactions between the positive zeta potential of the nanoparticle surface and the negative surface charge of the bacterial wall, penetrate the cell, and then induce oxidative stress by the aforementioned methods *(Thit, et al., 2015)*. In fact, the final ring free radicals and the formation of reactive oxygen species cause oxidative stress, which is proposed as a mechanism for the antimicrobial effect of metal nanoparticles. In addition, the presence and passage of nanoparticles through the bacterial membrane at high concentrations also lead to physical damage and loss of membrane integrity and continuity, leading to the destruction of the microorganism. Despite numerous studies on the effect of various nanoparticles, including silver nanoparticles, on fungal pathogenic strains, the mechanism and nature of the antifungal effect of nanoparticles have not been fully elucidated. In most studies on the mechanism of the antifungal properties of nanoparticles, it has been noted that in fungal cells, ergosterol present in the membrane, along with other proteins regulating ionic balances, plays a fundamental role in maintaining the stability and integrity of the membrane. Regulating a concentration gradient for various substances and compounds on both sides of the fungal cell membrane guarantees survival and proliferation through sporulation and the production of resistant spores. Therefore, it is believed that metal nanoparticles, by penetrating, the cytoplasmic membrane, and disrupting the concentration gradient of substances on both sides of the membranes, lead to metabolic disorders and ultimately to the death of the fungal cell *(Thit et al., 2015; Saison et al., 2011)*.

LITERATURE REVIEW

Recently, bacteria have become increasingly resistant to antibiotic treatment. However, the rate of development of more potent antibiotics to replace them has not kept pace with the rapid increase in bacterial resistance. It is also essential to develop new approaches to combat pathogenic bacteria and infections. Another way to fight infectious agents is to continually replace safe antibacterial agents, preventing different types of microbes from becoming resistant to them. Among these antibacterial agents, metal nanoparticles can be mentioned, which have been widely investigated and studied in recent years (M. Selvarani, et al., 2013). Recently, among the nanoparticles, the most attention has been paid to the copper nanoparticles. Cheap nanoparticles are proposed as alternatives to expensive metal nanoparticles in microelectronic applications and are likely the latest antimicrobial materials discovered (Sondi I., et al., 2004). In the present era, the antibacterial properties of copper oxide nanoparticles on gram-positive and gram-negative pathogenic bacteria that pose a threat to public health have been determined and established (Victor, et al., 2013).

In addition to the chemical properties of nanoparticles, two key features — high zone-to-mass proportion and size — have unexpectedly contributed to their properties. The smaller the nanoparticles, the greater their damage to bacterial power, which has given essential applications, including the catalytic role of nanoparticles (Azam, et al., 2012). Copper oxide metal nanoparticles have special physical-chemical properties that are due to the effect of quantum size and large effective surface area, which makes them different from the bulk state (Zhanhu Guo, et al., 2008). Copper oxide is the simplest member of the family of copper compounds that is effective on the bacterial cell membrane and can cause membrane rupture, resulting in the bacterial cell disintegrating and being destroyed. Carboxylamine groups and the peptide and glycan layer are the basic ingredients for the wall, respectively, of bacteria that react with Cu, causing injury at the cell septum, which is said to be one of the antimicrobial mechanisms of nanoparticles [Bogdanovića, et al, 2014]. It is worth noting that the mechanism of induction of antibacterial activity of metal oxide nanoparticles is not fully understood, but the amount of ion release and subsequent production of oxygen-free radicals is considered the only main reason for it (Beranva et al., 2014; Huh et al., 2011).

The present study sought to provide a positive answer to the anticipated question, "Do biosynthetic copper oxide nanoparticles exhibit antibacterial properties? Can these copper oxide nanoparticles be used as antimicrobial compounds for the control and treatment of pathogens such as B. cereus and P. aeruginosa?" An attempt was made to clarify this goal in a review study.

In 2014, Shantkriti and Ran succeeded in producing copper nanoparticles using the cell-free fluorescent medium of the non-pathogenic bacterium *Pseudomonas fluorescens*. The nanoparticles produced in this study were about 49 nm in size. They were spherical and showed antimicrobial effects *(*Sarwar S, et al., 2017*)*. In 2015, Ghorbani and his colleagues prepared CuONPs with a size of less than 10 nanometers using an extract prepared from *E. coli* bacteria. Scanning electron microscopy (SEM) studies revealed that the nanoparticles had a lattice structure *(Ghorbani, et al., 2015)*. In 2017, Ghasemi and his colleagues produced CuO and Ag/CuO nanoparticles with a size smaller than 10nm for the first time by the bacterium *Morganela morganii*. The nanoparticles produced in this study exhibited good antibacterial properties against the Gram-negative bacterium *P. aeruginosa* and the Gram-positive bacterium *B. cereus (Ghasemi, et al., 2017)*. In 2017, Khatami and his colleagues succeeded in producing copper oxide nanoparticles by an environmentally friendly method using the extract of the bacterium *Stachys Lavandulifolia*. The nanoparticles produced in this study had dimensions of about 80nm and showed highly damaging effects on bacterial properties, opposite for the gram-negative bacteria *P. aeruginosa (Khatami et al.,2017).*

METHODLOGY

Bacterial Cultivation and Its Conditions

The bacteria used included gram-positive *B. cereus* (PTTC1154) and gram-negative *P. aeruginosa* (PTTC1707) from Hazrat Seyed Al-Shohada Hospital in Urmia. They were cultivated in Blade agar-agar culture surroundings incubation for twenty-four hours at 37°C in the Biotechnology Laboratory of Ferdowsi University of Mashhad, Iran.

Preparation of Nanoparticles for Antibacterial Activity Testing

To investigate the antibacterial activity of copper oxide nanoparticles, 5g of nanoparticle powder was mixed with 50 ml of water, distilled three times, and after 24 hours, filtration was performed. After purification and desolvation of the resulting nanoparticle solution, concentrations of 40, 80, 160, 320, and 640 mg/ml were prepared with 10% DMSO solvent for use in well diffusion tests and MIC determination. The resulting solutions were stored at 5°C.

Investigation of Antibacterial Properties

The tested bacterial strains were revived using Luria-Bertani and Sabouraud dextrose culture media according to standard methods. In order to prepare a bacterial suspension, each bacterium was inoculated separately into test tubes containing 3 ml of Molar Heidon broth from a 24-hour culture, and a suspension with a turbidity equivalent to 0.5 McFarland (1.5×10^8 CFU/ml) was prepared.

To investigate the antibacterial effects of synthesized copper oxide nanoparticles, well diffusion methods and MIC determination were used. The series of runs used to determine the diameter of the bacterial growth inhibition zone in well diffusion methods was 5, 10, 20, 40, 80, and 160 µg/ml, and a solution of 40 µg/ml copper nitrate and the antibacterial antibiotic gentamicin was considered as a positive control. For to determine the MIC, a series of 10 tubes of 0/62, 1/25, 2/5, 5, 10, 20, 40, 80, 160 and 320 microgram/milliliter from CuONPs were used for each bacteria, and caption affirmative verification, a spout having solely the cultivation medium with an inoculated bacterium was used, and For negative control and to ensure the sterility of the work steps and the suspension of synthesized copper oxide nanoparticles, only the culture medium was added to one tube. In another tube, the culture medium containing a solution with a concentration of half a microgram per milliliter of copper oxide nanoparticles was added. The other steps of the work were the same as the preparation of the culture media of the bacterial strains used and the methods were similar to the techniques used in evaluating the potential for producing copper oxide nanoparticles.

RESULTS

Investigation of antibacterial activity of copper oxide nanoparticles synthesized by the gram-negative bacteria *Escherichia coli*

The results of the functional-antibacterial properties of biosynthetic CuONPs by Gram-negative bacteria, *Escherichia coli*, using well diffusion and MIC determination methods are shown in Tables(1) and (2), respectively. The synthesized nanoparticles showed a significant bactericidal effect on the tested strains, preventing the growth of bacteria at very low concentrations. It should be noted that the microbicidal impression of CuONPs is greater compared to other nanoparticles. Related results show that copper oxide nanoparticles had the most significant effect on *Pseudomonas aeruginosa* (20 mm halo diameter and 5 µg/mL MIC) and had the least impact on *Bacillus cereus* (17 mm halo diameter and 10 µg/mL MIC).

Table (1) Diameter of the bacterial growth inhibition zone at different concentrations of copper oxide nanoparticles by the well diffusion method (in millimetres)

Bacteria	**Various viscosities of CuONPs** (µg/ml)							
	5	10	20	40	80	160	**salt solution µg/ml**	**Gentamicin µg/ml**
Bacillus cereus	4	6	8	10	13	17	8	23
Pseudomonas aeruginosa	5	7	10	12	16	19	10	24

Table No. (2) Minimum Inhibitory Concentration (MIC) of Bacterial Growth at Different Concentrations of Copper Oxide Nanoparticles in (micrograms/mL)

Bacteria	Various viscosities of CuONPs (micrograms/mL)									
	0.62	1.25	2.5	5	10	20	40	80	160	320
Bacillus cereus	+	+	+	+	-	-	-	-	-	-
Pseudomonas aeruginosa	+	+	+	-	-	-	-	-	-	-

DISCUSSION

Since nanoparticles are a bridge between the bulk state of matter and the atomic and molecular state, they have attracted attention *(Kaushikthakkar N, et al., 2010)*. Metal nanoparticles have been extensively studied due to their interesting electrical, optical, chemical, and magnetic properties *(Azizi, at al., 2016)*.

Das and his colleagues have investigated the antimicrobial properties of biosynthetic CuONPs in various microorganisms, *including Pseudomonas aeruginosa,* *Bacillus circulans BP2, Escherichia coli,* and *Staphylococcus aureus*. They have concluded that these nanoparticles possess the ability to kill and control the growth of microbes. This is because increasing the concentration of CuO causes a defect in the function of cell enzymes and inhibits and even stops their growth rate *(Das, et al., 2013).* Efforts have been made to synthesize chelated CuNPs to achieve more stable materials. The Chelated products are more resistant and can be surrounded by ions from organic molecules (chelating agent) keep the chelating agent *(Chatterjee, et al; 2014)*. The amino acid–copper chelates, compared to copper nanoparticles, have shown tenfold antimicrobial activity *(DeAlba-Montero, et al., 2017).* Antimicrobial activity of copper nanoparticles has been demonstrated against various bacterial species including *B. subtilis*, Methicillin Resistant *s. Aureus* (MRSA) and also Gram-negative bacteria like *Pseudomonas aeruginosa*, *s. cholera*, and fungus genus like *C. albicans (Wang, et al., 2017).* Copper nanoparticles have been used as a source of methionine due to their microbial impact on tissues (*Chatterjee et al., 2014).*

In the study, the antibacterial activity of copper oxide nanoparticles synthesized by the gram-negative bacteria like *Escherichia coli* was tested, and it was also found that *Pseudomonas aeruginosa, Bacillus cereus*, and copper oxide nanoparticles had very high antibacterial effects at the relevant concentrations. Of course, the antibacterial activity was concentration-dependent. The resistance patterns of bacteria to copper oxide nanoparticles showed greater sensitivity of gram-negative bacteria than gram-positive ones, so that at low concentrations (5 μg/ml), gram-positive bacteria grew and were resistant, unlike gram-negative ones. Of course, with increasing concentration (10 microgram/milliliter), the germination of two bacteria remained. Additionally, the impact of CuONPs on bacterial activities has been investigated and demonstrated in numerous studies targeting various human pathogenic microbes.

In this study, gram-negative bacteria were found to be more sensitive, whereas gram-positive bacteria were less sensitive. The higher sensitivity of gram-negative bacteria to CuONPs can be attributed to the structural differences in their cell walls, which are thinner, resulting in lower wall strength. The outer surface of this wall also has a negatively charged (LPS) layer that plays a role in creating a strong interaction with CuONPs rich in charge. The attachment of nanoparticles to the cell surface first pierces the wall, and then, with the entry of the nanoparticles into the bacterial cell and interference in various metabolic and reproductive pathways, ultimately leads to the death of the bacteria. In general, the results of this study showed high antibacterial activity of pyrosynthetic copper oxide nanoparticles on the tested bacteria; therefore, their use in various fields is suggested, especially for prophylaxis from contamination and infection by pathogenic bacteria. In this study by Azam et al., the MBC value of copper oxide nanoparticles against *B. subtilis, S. aureus, P. aeruginosa*, and *E. coli* was reported to be 20, 22, 25, and 28, respectively, which showed lower antimicrobial effects compared to the nanoparticles produced in the present study *(Ren, et al., 2009).*

CONCLUSION

The high efficiency of various nanoparticles all over the world, especially in Afghanistan, requires more comprehensive and complete studies to simulate animal models and users in eukaryotic cells regarding this impression of the CuONPs on top various systems of at human body, and considering the importance of copper nanoparticles in the present era, investigating the effects of these particles on the human body system is of great significance and requires further research. The present study only examined one of the effects of these particles, which is the opposed bacterial properties from CuONPs synthesized with Gram- negative *E. coli*, on *Pseudomonas aeruginosa* and *Bacillus cereus* bacteria, and other essential characteristics of copper oxide nanoparticles such as determining their biological fate in vivo, toxicity with these materials, and their ability to target them. It is clear that to achieve the desired effects of using these nanoparticles in bucarbohydrates, further experiments and rigorous research are necessary.

REFERENCES

Azam, A., et al., .(2012). Antimicrobial activity of metal oxide nano particles against Gram-positive and Gram-negative bacteria a comparative study. International *Journal of Nanomedicine*. 6003-6009.

Azizinshermeh, O., Valizadeh, J., Noroozifar, M., Ghasemi, A., & Valizadeh, M. (2016). Optimization, characterization and antimicrobial activity of gold nanoparticles biosynthesized using aqueous extract of Sambucus ebulus. L". *Phytochem J Med Plants*, (1), 1-18. (Persian).

Beranva, J., Seydlova, G., Kozak, H., Benada, O., Fiser, R., Artemenko, A., Konopasek, I., & Kromka, A. (2014). Sensitivity of bacteria to diamond nanoparticles of various size differs in gram-positive and Gram- negative cells. *FEMS Microbiol. Lett.* dio:http://dx.doi. org /10.1111/1574-1111/ 6968.

Bogdanovića, U., Lazić, B. V., & Vodnik, V. (2014). Copper nanoparticles with high antimicrobial activity. *Materials Letter.*

Borkow. G., & Gabbay, J. (2005). Copper as a biocidal tool. *Current medicinal chemistry*,12 (18), 2163-2175.

Chatterjee, A. K., Chakraborty, R. & Basu, T. (2014). Mechanism of antibacterial activity of copper nanoparticles. *Nanotechnology Journla*, **25** (13), 135-101.

Das D., Nath B. H., Phukon, P., & Dolui, S. K. (2013) Synthesis and evaluation of antioxidant and antibacterial behavior of CuO nanoparticles *Colloids and Surfaces B: Biointerfaces*, (101), 430– 433.

De Alba-Montero, I., et al., .(2017). Antimicrobial properties of copper nanoparticles and amino acid chelated copper nanoparticles produced by using a soya extract. *Bioinorganic chemistry*, 217.

Ghasemi N, Jamali-Sheini F, Zekavati RJML (2017). CuO and Ag/CuO nanoparticles, *Biosynthesis and antibacterial properties, (*19), 78-82.

Ghorbani, HR., Fazeli, I., & Fallahi A.C. (2015). Biosynthesis of copper oxide nanoparticles using extract of E. coli. 2015;31(1), 515-7.

Gold, K., Slay, B., Knackstedt, M., & Gaharwar, AK.(2018). Antimicrobial Activity of Metal and Metal-Oxide Based Nanoparticles. Advanced Therapeutics,1(3),170-133.

Hajipour, MJ, et al.(2012). Antibacterial properties of nanoparticles. *Trends in Biotechnology*. 30(10):499-.

Huh, A.J. & Kwon, Y.J. (2011). Nanoantibiotics: a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. *Journal of Control. Release* 156, 128–145.

Kaushikthakkar N, Snehitmhatre S, Raseshparikh, Y. (2010). Biological synthesis of Metallic Nanoparticles. Nanomedicine, *Trends in Biotechnology*. (28), 257-262.

Khatami, M., Heli, H., Jahani, PM., & Azizi, H.(2017).Copper oxide nanoparticles synthesis using Stachys lavandulifolia and its antibacterial activity. *Trends in Biotechnology*, 11(6),709-723.

Selvarani, M., & Prema, P. (2013). "Evaluation of antibacterial efficacy of chemically synthesid copper and zerovalent Iron nanopartical", *Asian Journal of Pharmaceutical and Clinicaleserch. (*9), 74-92.

Mandal, D., Bolander, ME., Mukhopadhyay D., Sarkar, G., & Mukherjee P. (2006). The use of microorganisms for the formation of metal nanoparticles and their application. *Applied microbiology and biotechnology*, 69 (5), 485-92.

Ren, G., et al. (2009). *Characterisation of copper oxide nanoparticles for antimicrobial applications.* International journal of antimicrobial agents, 33 (6), 587-590.

Saison, C., et al. (92010). Effect of core–shell copper oxide nanoparticles on cell culture morphology and photosynthesis (photosystem II energy distribution) in the green alga, *Chlamydomonas Reinhardtii. Aquatic Toxicology*. 96(2),109-14.

Sarwar, S., Ali, A., & Pal, M. (2017). Zinc oxide nanoparticles provide anti-cholera activity by disrupting the interaction of cholera toxin with the human *GM1 receptor*, 29 (44), 303-311.

Sharma D, Kanchi S, Bisetty K. Biogenic synthesis of nanoparticles: A review. Arabian journal of chemistry. 2015.

Sondi I., & Salopek-Sondi, B. (2004). Silver nanoparticles as antimicrobial agent. *Journal of Colloid Interface Science*. (27),177-182.

Thakkar, KN., & Mhatre, SS .(2010). Biological synthesis of metallic nanoparticles, 6(2), 57-62.

Thit, A., Selck, H., & Bjerregaard, HF. (2015). Toxic mechanisms of copper oxide nanoparticles in epithelial kidney cells. *Toxicology in Vitro*., 29(5),105-119.

Victor, K., Dennis., J., & Helfritch, A.(2013). Demonestration of the antimicrobial effectiveness of various copper surfaces, Champagne and Helfritch, *Journal of Biological Engineering* (7), 1-8.

Wang, L., Hu, C., & L. (2017). Shao, *The antimicrobial activity of nanoparticles: present situation and prospects for the future. International Journal of Nanomedicine,* (**12**), 1227.

William, F., Smith & Hashemi, J. (2011). Foundations of materials science and engineering. *McGraw-Hill*

Zhanhu Guo et al., (2007). Strengthening and thermal stabilization of polyurethane nanocomposites with silicon carbide nanoparticles by a surface-initiated polymerization approach composites. *Composites Science and Technology,*(68)164-170.

Zhao, J., Wang, Z., Liu, X., Xie, X., Zhang, K., & Xing, B.(211). Distribution of CuO nanoparticles in juvenile carp (Cyprinus carpio) and their potential toxicity. Journal of hazardous materials.;19, (7), 304-10.

Lewinski, N., Colvin, V., & Drezek, R. (2008). Cytotoxicity of nanoparticles. *Small.*4(1), 26.