

Antibacterial effects of copper- and silver-coated carbon nanotubes synthesized by plasma-enhanced chemical vapor deposition on *Staphylococcus aureus* and *Escherichia coli*: a comparative study

Pooya Sepehr¹ , Seyed Majid Borghei^{2*} , Morad Ebrahimkhas¹,
Nasim Nobari¹

¹Department of Physics, Mahabad branch, Islamic Azad University, Mahabad, Iran.

²Department of Physics, Karaj branch, Islamic Azad University, Karaj, Iran.

*Corresponding author: majid.borghei@kia.ac.ir

Original Research

Abstract:

Received:

24 July 2024

Revised:

1 September 2024

Accepted:

8 September 2024

Published online:

15 December 2024

© The Author(s) 2024

The use of copper (Cu) and silver (Ag) nanoparticles in coatings can eliminate surface microbial contamination. This study compared antibacterial activity of Cu- (Cu/CNTs) and Ag-coated carbon nanotubes (Ag/CNTs) synthesized by plasma-enhanced chemical vapor deposition (PECVD) against *Escherichia coli* and *Staphylococcus aureus*. Initially, the PECVD technique was applied to deposit the CNTs on high-resistivity silicon wafers previously decorated by nickel catalyst using an Electron Beam Gun. Then, the nanotubes were coated by Cu and Ag thin films in a vacuum evaporator using the Direct Current (DC) Magnetron Sputtering method. Finally, the antibacterial effects were determined by Standard Plate Count (SPC, with film thicknesses of 0, 10, 30 and 60 nm) and Disk Diffusion Test (based on zone of inhibition (ZOI) with nanoparticle concentrations of 5, 10 and 15 $\mu\text{g/mL}$). According to the SPC findings, the highest antibacterial activity of Cu/CNTs was found for the film thickness of 60 nm against *E. coli* (66%), and the lowest activity was related to the film thickness of 19 nm against *S. aureus* (28.8%). The antibacterial activity of Ag/CNTs was about 70% against *E. coli* with the highest thickness and about 34.12% against *S. aureus*. The lowest ZOI was measured for the bare CNTs at a concentration of 5 $\mu\text{g/mL}$ (12 mm), and the highest ZOI was related to Ag/CNTs with a concentration of 15 $\mu\text{g/mL}$ against *S. aureus* (18 mm). To conclude, the carbon nanotube composites coated with copper or silver nanoparticles can be used to control bacterial growth in aqueous solutions.

Keywords: Antibacterial activity; Copper-coated carbon nanotubes; Silver-coated carbon nanotubes; Plasma-enhanced chemical vapor deposition; *Staphylococcus aureus*; *Escherichia coli*

1. Introduction

Today, microbial contamination of surface water resources and various surfaces has become a serious global public health concern (Khoramnejadian and Fatemi, 2016). One of the current challenges confronting surface water resources is their contamination with fecal coliforms that endanger the life of all living organisms (Saberinia et al., 2021). New bacterial strains have been emerging in the past several decades,

which are resistant to common antibiotics and antimicrobial agents, thus researchers are looking for suitable and cost-effective solutions to bypass this bottleneck (Orhan et al., 2021). The rate of growth and spread of these strains has been faster than the rate of production of effective antibiotics and antibacterial substances (Yun et al., 2013). There are different methods to remove microbial contaminants. Considering the environmental problems at the global level, environmentally friendly approaches have always at-

tracted special attention. A solution is environmentally friendly during which the production of greenhouse gases is minimized and hazardous by-products are not produced (Ohadian et al., 2023; Fekri et al., 2021). Some natural polymers and elements have antibacterial properties (Khoramnejadian, 2011; Aloucheh et al., 2024). In this context, the use of nanoparticles (NPs) has been considered in order to reduce or eliminate pollutants (Moghanjooghi et al., 2022). It has been reported that NPs have the ability to remove toxins and organic pollutants (Khodkar et al., 2019). A wide range of carbon nanostructures (CNS) has been produced for various applications, such as carbon nanotubes (CNTs), nanodiamonds, fullerenes, nanofibers and other carbon-based nanomaterials (Saleemi et al., 2022). Currently, CNTs are widely used in the development of antimicrobial surfaces. The antimicrobial activity of CNTs depends on various factors, including composition, length, size, number of graphene layers, and physical distribution (Chen et al., 2013). The antimicrobial activity and other satisfactory applications of CNTs are due to their commendable physicochemical and structural properties, such as purity, diameter, length, surface functional groups and electronic structure. According to research findings, the smaller the diameter and the shorter the length of CNTs, the higher their antibacterial activity (Li et al., 2011). The interaction of CNTs with biological systems can induce some cytotoxic effects, such as reactive oxygen species (ROS) generation, hypersensitivity, DNA damage and protein dysfunction (Shvedova et al., 2012). The CNTs have a mesopore-like structure with a high potential to eliminate organic substances, and have a higher ability to absorb pollutants due to the charge of the functional groups (Tehrani and Skandari, 2023).

Silver nanoparticles (AgNPs) have been used in medicine to control bacterial growth (Catauro et al., 2004). They have been exploited in biotechnology and life sciences for research in various fields, including inflammation, drug delivery, burns treatment and biochips (Seo et al., 2014). Ionic and Nano silver particles have disrupted photosynthesis and cell function (Balandeh et al., 2022). Silver particles not only have shown antibacterial properties, but also have had destructive effects on fungi and viruses (Furno et al., 2004). The use of inorganic nanoparticles compared to other antibacterial chemicals has a unique advantage, so that the chemicals induce drug resistance in bacteria, but the inorganic particles adhere to and destroy cell wall without causing drug resistance (Mollania et al., 2016). Studies have shown the antibacterial effect of silver ion on 12 bacterial strains, including *Escherichia coli* (Orhan et al., 2021). *E. coli* is a gram-negative bacterium found in the digestive system of warm-blooded organisms. The presence of *E. coli* (fecal coliform) in water is a strong indicator of sewage or animal waste contamination (Osouledini et al., 2024). A study showed that the growth of *E. coli* was controlled to a large extent at AgNPs concentration of 13.2 nm (Liu et al., 2006). The use of AgNPs as an antibacterial agent is more effective and cost-effective than larger silver particles due to the special and unique properties of nanomaterials. Silver is a chemical element with low toxicity, high thermal stability

and high antibacterial activity (Hamouda et al., 2021). Due to the lack of stability of AgNPs and to prevent the reduction of their antibacterial properties, they are coated with materials such as silicate compounds and zeolites or are involved in the formation of composites with CNTs (Kazmi et al., 2014). Incorporation of silver or copper ions to CNTs can enhance the antibacterial activity (Kim et al., 2013). Covalent bonding occurs in silver-coated multi-walled carbon nanotubes. The solubility of multi-walled carbon nanotubes is increased by covalent bonding, which is the result of the effect on Van der Waals Forces (Tarlani et al., 2015). Previous studies reported different intensity of activity for silver (AgNPs) and copper (CuNPs) nanoparticles against different bacterial strains. CuNPs have been effective against *E. coli* and also had better performance against bacilli compared to AgNPs (Raffi et al., 2010). CuNPs have been successful in the elimination of organic pollutants (Fekri et al., 2023).

This study aimed to compare the antibacterial activity of Cu/CNTs and Ag/CNTs synthesized by plasma-enhanced chemical vapor deposition (PECVD) technique against two strains of gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacteria.

2. Materials and methods

2.1 Preparation of the substrate

One-inch slices of silicon wafer, <100>, type P, were used to prepare the substrate. It is resistant to high temperatures. The prepared slices were completely cleaned of any pollution. Thus, they were washed first with water and then with acetone-alcohol solution for 10 minutes with an ultrasonic device. At last, the substrates were dried under nitrogen gas.

2.2 Preparation of the catalysts

Transition metal catalysts were needed for the growth of CNTs in the PECVD method, because the absence of such metals could lead to the production of amorphous carbon during the process. The size and diameter of the catalyst particles have a relationship with the produced nanotubes (Choi et al., 2000). In this work, nickel (Ni) was considered as a catalyst. There would be no growth in the absence of this catalyst. For each sample, a thin Ni film (9 nm), as a catalyst, was deposited on the silicon wafer substrate using an electron beam gun at a temperature of 120 °C and a deposition rate of 0.01 nm/s.

Then, the samples were placed in the quartz tube of the DC-PECVD system (Model: SensIran PE-802). The process of nanotube growth was performed in this system; first, the substrate sample prepared in the reactor was placed on the negative pole of the plasma and the vacuum conditions were established in the system to remove excess gases. In this case, the system vacuum was under pressure. The temperature of the sample was brought to 650 °C by the device heater and kept constant for 15 minutes along with hydrogen gas blowing at a flow rate of 20 sccm. Setting the device at the optimal temperature prevented the generation of undesirable SiZOH bonds (Pereyra and Alayo, 1997). Thus, the nano-sized Ni islands were on the verge of for-

mation (Nasehnia et al., 2004). By turning on the power of Direct Current Plasma (DCP), this step was continued by applying hydrogen plasma (with a current of 30 mA and a voltage of 500V) for 5 minutes at the same temperature (650 °C). Thus, the Ni atoms earned the required kinetic energy to merge with each other. The Ni ions entered the amorphous Ni films and made it crystallized. Therefore, the nanometer islands were formed under suitable temperature and plasma conditions. Acetylene gas (C₂H₂) was introduced into the system at a flow rate of 5.4 sccm as a source of carbonization. Current and voltage in this status were 32 mA and 600 V, respectively, and the optimal contact time of CNTs was within 15 to 25 minutes. These conditions appeared to have provided the energy necessary to break the carbon-carbon bond. The carbonation of the system was tested at different times and it was found that increasing the contact time caused the size of the nanotubes to change, and thus the contact time was limited to 15 to 25 minutes to achieve the desired growth of CNTs. After the contact time, acetylene gas was stopped entering the system, and the temperature was lowered to 150 °C without stopping the flow of hydrogen gas. At this stage, the growth process ended and the sample was slowly harvested from the system.

2.3 Copper and silver coating on the carbon nanotubes

At this phase, the copper and silver thin films were deposited on the grown CNT substrates at different times using a DC magnetron sputtering system (Model EDS-160). First, the device was cleaned of any pollution. Copper and silver ions, as the target materials, were separately introduced into the system and then the substrates on which CNTs had grown were attached to the sample holders and placed inside the system. The system pressure reached up to 6.2×10^{-5} mbar by the vacuum pump, and then the vacuum pressure reached up to 10^{-2} mbar by the rotary pump. In the next step, argon gas was introduced into the chamber, which caused the pressure to increase up to 5×10^{-5} mbar; applying voltage to the cathodes (the target materials) caused the formation of an argon gas plasma environment. The introduction of argon gas caused the existing vacuum to be broken to a pressure of 10^{-2} mbar. To deposit different thicknesses of copper

and silver, each of the samples (substrates) was placed in the anode position for different periods of time by rotating the holders. The thickness was measured by a quartz crystal system and displayed on a monitor (Nasehnia et al., 2004). Table 1 shows the general conditions of deposition. The structure and morphology of the samples were characterized by SEM and FE-SEM (Model: Sigma, ZEISS company, Germany; DES detector: Oxford Instruments, UK) and TEM (Model: EM10C-100KV, ZEISS company, Germany; Tecnai 20: FBI company). After confirming the formation of nanotubes-nanoparticles, antibacterial studies were performed.

2.4 Preparation and cultivation of the studied bacteria

The two bacterial strains of *Staphylococcus aureus* (ATCC 6538, PTCC 1112) and *Escherichia coli* (ATCC 25922, PTCC 1399) were prepared from Pasteur Institute of Iran and then tested for the antibacterial effects of as-synthesized Ag/CNTs and Cu/CNTs by Standard Plate Count (SPC, with film thicknesses of 0 (control), 10, 30 and 60 nm, in CFU/mL) and Disk Diffusion Test (based on zone of inhibition (ZOI) with nanoparticle concentrations of 5, 10 and 15 µg/mL, in mm) (Kalyana-Sundaram et al., 2012). All experiments were performed in triplicate. In the SPC method, the number of counted colonies (in CFU/mL) was also reported as antibacterial activity (k , in percentage), according to Equation 1.

$$k = \frac{(A - B)}{A} \times 100\% \quad (1)$$

where, A refers to the bacterial count related to reference sample (bare CNTs) and B refers to the bacterial count related to silver/copper-coated CNTs.

2.5 Anti bacterial test in liquid media

For bacterial culture, a loop of pure bacterial colonies was transferred into Luria-Bertani nutrient broth (LB-Merck) (meat extract and peptone) and the samples were homogenized using a shaker. The samples were incubated for 24–48 h under aerobic conditions at 25–30 degrees Celsius in a shaking incubator aimed at promoting bacterial growth. The samples were then centrifuged at a speed of 5000 rpm for 20 min (Kalyana-Sundaram et al., 2012). Samples were diluted to pH 7.2 to achieve a concentration of 20 µg/ml for experiments. Wells of 6–8 mm diameter were created on the medium and microbial agents were added. 20 microliters of bacterial suspension in 7.2 pH phosphate buffer were injected into 1 ml of samples into containers containing carbon nanotubes containing copper and silver nanotubes. The effectiveness of the antibacterial agent was determined by measuring the aura around the wells. Bacterial colonial growth inhibition was investigated at different concentrations of carbon nanotubes associated with copper and silver nanoparticles.

The concentrations of 5, 10 and 15 µg/ml were used. Experiments were carried out in 3 repetitions.

3. Results

Table 1 shows the number of counted colonies of two bacterial strains exposed to Cu/CNTs at different film

Table 1. The number of counted colonies of two bacterial strains exposed to copper-coated carbon nanotubes (Cu/CNTs) at different film thicknesses based on the SPC method.

Bacterial strains	Cu/CNT samples (nm)	Bacterial count (CFU/ml) $\times 10^7$	Antibacterial activity (%)
<i>E. coli</i> ATCC 25922	60	1.7	66
	30	1.8	64
	10	3.3	34
Control	0	5	-
<i>S. aureus</i> ATCC 6538	60	2.0	55.56
	30	3.0	33.33
	10	3.2	28.89
Control	0	4.5	-

Table 2. The number of counted colonies of two bacterial strains exposed to silver-coated carbon nanotubes (Ag/CNTs) at different film thicknesses based on the SPC method.

Bacterial strains	Cu/CNT samples (nm)	Bacterial count (CFU/ml) $\times 10^7$	Antibacterial activity (%)
<i>E. coli</i>	60	1.7	70
ATCC 25922	30	1.8	66
	10	3.3	39
Control	0	5	-
<i>S. aureus</i>	60	2.0	64.21
ATCC 6538	30	3.0	41.31
	10	3.2	34.12
Control	0	4.5	-

thicknesses based on the SPC method. According to the findings of SPC method, the highest antibacterial activity of Cu/CNTs was related to the film thickness of 60 nm against *E. coli* (66%), and the lowest activity was related to the film thickness of 19 nm against *S. aureus* (28.89%).

Table 2 shows the number of counted colonies of two bacterial strains exposed to Ag/CNTs at different film thicknesses based on the SPC method. According to the findings of SPC method, the antibacterial activity of Ag/CNTs against *E. coli* was higher than that of against *S. aureus*. Moreover, the antibacterial activity of Ag/CNTs was about 70% against *E. coli* with the highest thickness and about 34.12% against *S. aureus*.

Figure 1 compares the antibacterial activity (%) of Ag/CNTs and Cu/CNTs against *E. coli*. The Ag/CNTs possessed more antibacterial efficiency. Figure 2 compares the antibacterial activity (%) of Ag/CNTs and Cu/CNTs against *S. aureus*. The activity of Ag/CNTs against gram-positive bacteria was higher than that of Cu/CNTs. Against *S. aureus*, Ag/CNTs with a film thickness of 60 nm had an antimicrobial activity of about 64%. The antimicrobial activity of Cu/CNTs in the same film thickness and conditions was about 55%, indicating the greater antimicrobial activity of Ag/CNTs. Figures 3 and 4 show the ZOI related to Cu/CNTs and Ag/CNTs at different concentrations created against *E. coli* and *S. aureus*.

Table 3 shows the mean ZOI diameter related to bare CNTs, Cu/CNTs and Ag/CNTs at three nanoparticle concentrations of 5, 10 and 15 $\mu\text{g/mL}$ created against *E. coli* and *S. aureus*. As can be seen, the smallest and largest ZOI diameters were related to bare CNTs and Ag/CNTs (with a particle concentration of 15 $\mu\text{g/mL}$) against *E. coli*. In addition, the lowest and highest ZOI diameters were measured for bare CNTs and Ag/CNTs (with a particle concentration of 15 $\mu\text{g/mL}$) against *S. aureus*.

4. Discussion

The number of counted colonies exposed to Cu/CNTs showed that the antibacterial activity was enhanced with increasing Cu film thickness. The antibacterial activity of

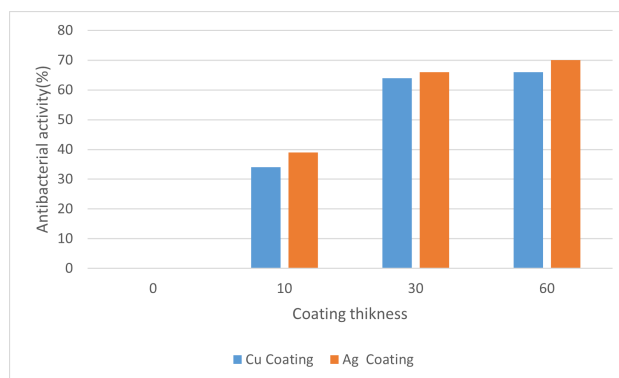


Figure 1. Comparison of antibacterial activity of Ag/CNTs and Cu/CNTs against *E. coli*.

Cu/CNTs at the film thicknesses of 10, 20 and 60 nm was determined to be about 66, 64 and 34% against *E. coli*, respectively. The mechanism of action of Cu ions has been determined to be penetration into the cell wall and membrane of bacteria, thus leading to cell disintegration. The Cu ions, which have a positive charge, bind to the free electrons of bacterial cells, causing cytoplasmic dysfunction and nuclear oxidation (Zhang et al., 2006). The thicker the Cu film, the more Cu molecules are detached from the surface and attack the bacteria. The bacterial membrane as a structural component is affected by biocidal challenges such as antibacterial substances and antibiotics (Meghana et al., 2015). The maintenance of intracellular components is the responsibility of the cell membrane. Following membrane damage, small ions such as potassium and phosphate tend to exit the cell body, followed by DNA and RNA molecules (Jr et al., 2008). The antibacterial activity of Cu/CNTs at the film thicknesses of 10, 20 and 60 nm was determined to be about 55, 33 and 28% against *S. aureus*, respectively. The Cu/CNTs and Ag/CNTs showed stronger antibacterial activity against *E. coli* compared to *S. aureus*. Studies have shown that compounds made from copper nanoparticles have antibacterial and antimicrobial properties, making them effective against pathogens such as *E. coli* and *Staphylococcus aureus* (Zhang et al., 2020). Copper nanoparticles have antibacterial effects against pathogenic microorganisms such as *E. coli*. Therefore, increasing the copper thick-

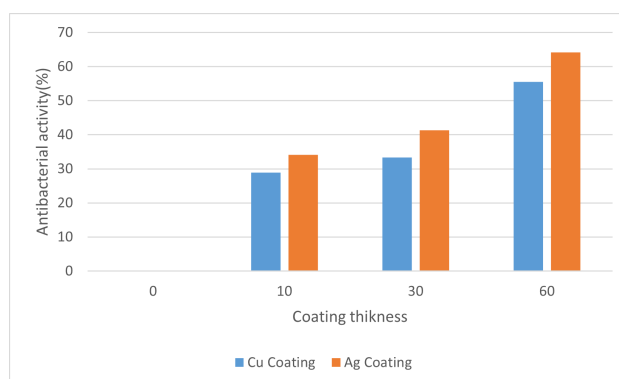
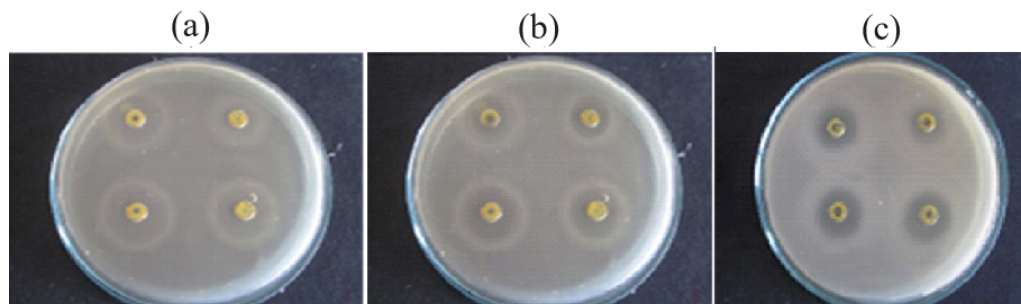
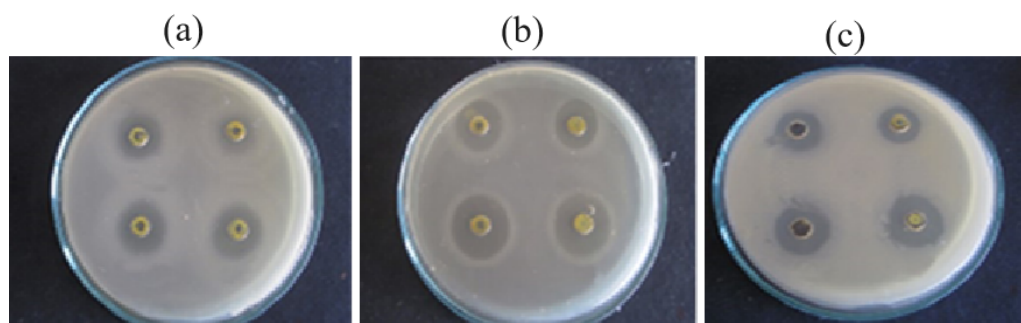


Figure 2. Comparison of antibacterial activity of Ag/CNTs and Cu/CNTs against *S. aureus*.

Table 3. The mean diameter of zone of inhibition (mm) related to bare CNTs, Cu/CNTs and Ag/CNTs at different concentrations created against studied bacteria.

Samples Concentration ($\mu\text{g/mL}$)	<i>E. coli</i> ATCC 25922			<i>S. aureus</i> ATCC 6538		
	5	10	15	5	10	15
Bare CNTs	8	9	11	12	13	13
Cu/CNTs	12	14	15	15	15	11
Ag/CNTs	13	15	17	16	17	18

**Figure 3.** The zone of inhibition (mm) related to Cu/CNTs at (a) 5 $\mu\text{g/mL}$ and (b) 10 $\mu\text{g/mL}$ (c) 15 $\mu\text{g/mL}$ concentration created against *E. coli*.**Figure 4.** The zone of inhibition (mm) related to Ag/CNTs at (a) 5 $\mu\text{g/mL}$ and (b) 10 $\mu\text{g/mL}$ (c) 15 $\mu\text{g/mL}$ concentrations created against *S. aureus*.

ness increases the antibacterial effect.

The antibacterial activity of Ag/CNTs at the film thicknesses of 10, 20 and 60 nm was determined to be about 70, 66 and 39% against *E. coli*, respectively. When conditions occur where the membrane is destroyed, the mechanism by which Cu and Ag destroy bacteria is a smart approach. According to one hypothesis, Cu reacts with endogenous hydrogen peroxide (H_2O_2) and produces hydroxide ions, which reacts with oxygen or hydroxyl radicals ($\cdot\text{OH}$), similar to the Fenton process (Lloyd and Phillips, 1999). The mechanism of action of ROS, which creates free radicals, is the reaction with and as a result the destruction of cell wall proteins (Nohegar et al., 2022). The biocidal action of CuNPs is based on the reaction of copper ions with thiols, as silver also has this feature. Amino acids present in bacterial cells tend to chelate Cu particles (Semisch et al., 2014). Silver nanoparticles are known for their many applications and remarkable properties such as large contact area and high antibacterial activity that prevents the formation of microbial communities (Antunes et al., 2023).

The antibacterial activity of Ag/CNTs at the film thicknesses

of 10, 20 and 60 nm was determined to be about 66, 64 and 34% against *S. aureus*, respectively. The outer membrane of gram-positive bacteria consists of peptidoglycan layer consisting of sugar and amino acids, and phosphoryl substituted with teichoic and teichuronic acids as well as carboxylate groups. In gram-negative bacteria, the cell membrane is composed of an additional layer of lipopolysaccharide (LPS) and phospholipids in addition to the peptidoglycan layer (Deokar et al., 2013). This additional protection may inhibit the bactericidal potential of the dispersed nanotubes against gram-negative bacteria. Cuo-Nano causes cell death and DNA damage. Ag-Nano does not produce toxicity. Cuo-Nano toxicity is primarily caused by intracellular absorption and secondarily by free copper ions. Ag-Nano does not exhibit the short-term toxicity of silver ions in the short term (Cronholm et al., 2013).

The analysis of the findings indicates the higher antibacterial potential of AgNPs compared to CuNPs. Studies have shown that Ag nanomaterials and Ag-containing products were effective against *E. coli* (Prodana et al., 2011). Based on previous research, the effect of AgNPs on *S. aureus* has

been greater (Marambio-Jones and Hoek, 2010). Nanoparticles exhibit different properties depending on their size, the various complexes of nanoparticles and metals act synergistically and affect antibacterial properties (Kot et al., 2023). It seems that Ag is making a better compound than Cu with CNTs.

Our results showed higher antibacterial activity of Ag/CNTs against *E. coli* compared to Cu/CNTs. The findings reveal the inhibitory potential of nanoparticles against bacterial growth and production. Ag particles cause morphological changes in the bacterial cell, so that the cytoplasm shrinks and thus the cell wall is destroyed and the cell contents leak out (Jung et al., 2009). A study reported the effect of different AgNPs concentrations on the structure of soil microbial communities, so that Ag particles had a decreasing effect on soil microorganisms (Samarajeewa et al., 2017). In our study, the antibacterial activity of Ag/CNTs at the film thickness of 60 nm against *E. coli* reached about 70%, which was a remarkable outcome. The results of measuring the antibacterial activity of Cu/CNTs against *E. coli* and *S. aureus* showed that the largest ZOI diameter within 24 hours was related to Ag/CNTs against *E. coli*. The ZOI diameter was reduced with the decrease in the concentration of CNTs, in line with the findings of other studies (Nohegar et al., 2022). The antibacterial activity of CuNPs is related to the adhesion of bacteria due to their opposite electric charges, leading to a reduction reaction in the bacterial cell wall (Raffi et al., 2010). According to the research, the CNTs had toxicity to *E. coli*, but had no mutagenic effect (Sotto et al., 2009). Multi-walled carbon nanotubes are more toxic than single-walled carbon nanotubes. Multi-walled carbon nanotubes, because they have active OH agents, are more dispersed in the colloidal state and have a greater inhibitory effect on bacteria (Joonaghani et al., 2019). The results demonstrated that magnetic Cu and Ag nanoparticles had a higher efficiency to remove *E. coli* (Zainalzadeh et al., 2015).

The data on the mean ZOI diameter for *S. aureus* showed that the largest ZOI diameter was related to Ag/CNTs (16mm). The ZOI diameter was reduced with decreasing nanoparticle concentration. The smallest ZOI diameter was related to bare CNTs at the concentration of 5 $\mu\text{g/mL}$ (12 mm). In general, nanomaterials release ions that react with thiol groups of membrane proteins. Cell membrane proteins play a role in cell wall permeability. Nanomaterials can inactivate these proteins, thus resulting in reduced membrane permeability and cell death (Stoimenov et al., 2003). In addition, they generate antibacterial compounds such as $\cdot\text{OH}$ and H_2O_2 (Shanthi et al., 2016). A study reported that nanoparticles could inhibit the growth of *E. coli* and *S. aureus* (Khani et al., 2011). The effectiveness of nanoparticles depends on the bacterial structure (gram -positive and gram -negative). The concentration of 15 ppm showed the strongest biocidal activity against *Staphylococcus aureus* G+ and *Escherichia coli* (G-). According to Alizadeh et al., Ag nanoparticles showed greater efficacy against bacteria at concentrations above 10 ppm.(Alizadeh et al., 2013).

5. Conclusion

In the current research, the carbon nanotubes (CNTs) synthesized by plasma-enhanced chemical vapor deposition (PECVD) were coated with silver (Ag/CNTs) and copper (Cu/CNTs) nanoparticles. Then, the antimicrobial activity of as-synthesized Cu/CNTs and Ag/CNTs were compared against two strains of gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacteria. The purpose of this research was to remove microbial contaminants, especially in aqueous solutions. The number of counted colonies exposed to bare and coated CNTs showed that the antibacterial activity increased as the thickness of the copper and silver films increased. The antibacterial activity of Ag/CNTs was higher than that of Cu/CNTs, which was attributed to the higher antimicrobial activity of silver particles. All the tests and results of this research revealed that PECVD-grown Cu/CNTs and Ag/CNTs had a satisfactory potential to eliminate microbial pollutants. Therefore, these composites are suggested to be used as a complementary process in water treatment.

Acknowledgments

This research has been extracted from the PhD dissertation in Physics written by Mr. Pooya Sepehr at the Islamic Azad University of Mahabad. Therefore, the authors appreciate the president, educational and research deputies of Mahabad Islamic Azad University for their cooperation in facilitating the implementation of this project.

Authors Contributions

Study concept and design: Pouya Sepehr, and Seyed Majid Borghei; analysis and interpretation of data: Morad Ebrahimkhas, and Nasim Nobari; drafting of the manuscript: Pouya Sepehr; critical revision of the manuscript for important intellectual content: Seyed Majid Borghei.

Availability of Data and Materials

All data generated or analysed during this study are available from the corresponding author upon reasonable request.

Conflict of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Open Access

This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative

Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the OICCPress publisher. To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0>.

References

- Alizadeh H., Salouti M., Shapouri R. (2013) Intramacrophage antimicrobial effect of silver nanoparticles against *brucella melitensis* 16m. *Sci. Iran.* 20:1035–3098.
- Aloucheh R. Mohammadi, Baris O., Asadi A., zadeh S. Gholam, sadeghi M. Kharat (2024) Characterization of aquatic beetles shells (hydraenidae family) derived chitosan and its application in order to eliminate the environmental pollutant bacterial. *Anthropog. pollut.* 3 (2): 43–48.
- Antunes K. E. K. G. R., Rocha D. N., Marcelino L., Nascimento Silva R. do, Nogueira A. P., Facury A. G. B. F., Neves J. G. (2023) Synthesis of an experimental gel containing biologically synthesized silver nanoparticles used in the biofilm disruption. *ARCHIVES OF HEALTH INVESTIGATION* 12 (7): 1482–1487.
- Balandeh S., Lakzian A., Javadmanesh A. (2022) Effects of silver nanoparticles on soil microbial activity and bacterial populations in a calcareous soil using qpcr. *Journal of Water and Soil.* 35 (6): 843–859.
- Catauro M., Raucci M. G., Gaetano F. D. De, Marotta A. (2004) Antibacterial and bioactive silver-containing $na_2o \times cao \times 2sio_2$ glass prepared by sol-gel method. *J. Mater. Sci.: Mater. Med.* 15:831–837.
- Chen H., Wang B., Gao D., Guan M., Zheng L., Ouyang H., Chai Z., Zhao Y., Feng W. (2013) Broad-spectrum antibacterial activity of carbon nanotubes to human gut bacteria. *Small* 9 (16): 2735–46. <https://doi.org/10.1002/sml.201202792>
- Choi Y. C., Shin Y. M., Lim S. C., Bae D. J., Lee Y. H., Lee B. S., Chung D. C. (2000) Effect of surface morphology of ni thin film on the growth of aligned carbon nanotubes by microwave plasma-enhanced chemical vapor deposition. *J. Appl. Phys.* 88 (8): 4898–4903.
- Cronholm P., Karlsson H. L., Hedberg J., Lowe T. A., Winnberg L., Elihn K., Moller L. (2013) Intracellular uptake and toxicity of ag and cuo nanoparticles: a comparison between nanoparticles and their corresponding metal ions. *Small* 9 (7): 970–982.
- Deokar A. R., Lin L. Y., Chang C. C., Ling Y. C. (2013) Single-walled carbon nanotube coated antibacterial paper: preparation and mechanistic study. *J. Mater. Chem. B.* 1:2639–2646.
- Fekri R., Mirbagheri S. A., Fataei E. (2021) Organic compound removal from textile wastewater by photocatalytic and sonocatalytic processes in the presence of copper oxide nanoparticles. *Anthropogenic Pollution* 5 (2): 93–103.
- Fekri R., Mirbagheri S. A., Fataei E., Ebrahimzadeh-Rajaei G., Taghavi L. (2023) Organic compound removal from textile wastewater by photocatalytic and sonocatalytic processes in the presence of copper oxide nanoparticles. *Anthropog. pollut.* 5 (2): 93–103.
- Furno F., Morley K. S., Wong B., Sharp B. L., Arnold P. L., Howdle S. M., Bayston R., Brown P. D., Winship P. D., Reid H. J. (2004) Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection. *J. Antimicrob. Chemother.* 54:1019–1024.
- Hamouda H. I., Abdel-Ghafar H. M., Mahmoud M. H. H. (2021) Multi-walled carbon nanotubes decorated with silver nanoparticles for antimicrobial applications. *J. Environ. Chem. Eng.* 9 (2): 105034.
- Joonaghani M. A., Tavabe K. Rezaei, Nezhadheydari H., Mirvaghefi A., Farhang P. (2019) Study of cnt@fe3O4 effects on aeromonas hydrophila and yersinia ruckeri bacteria isolated from fish. *J. Fish.* 72 (1): 57–67. <https://doi.org/10.22059/jfisheries.2019.74390>
- Jr A. A. Santos, Ferket P. R., Santos F. B. O., Nakamura N., Collier C. (2008) Change in the ileal bacterial population of turkeys fed different diets and after infection with salmonella as determined with denaturing gradient gel electrophoresis of amplified 16S ribosomal dna. *Poultry science* 87 (7): 1415–1427.
- Jung R., Kim Y., Kim H. S., Jin H. J. (2009) Antimicrobial properties of hydrated cellulose membranes with silver nanoparticles. *journal of biomaterials science. J. Biomater. Sci. Polym. Ed.* 20:311–332.
- Kalyana-Sundaram S., Kumar-Sinha C., Shankar S., Robinson D. R., Wu Y. M., Cao X., Asangani I. A., et al. (2012) Expressed pseudogenes in the transcriptional landscape of human cancers. *Cell.* 149 (7): 1622–1634. <https://doi.org/10.1016/j.cell.2012.04.041>.
- Kazmi S. J., Shehzad M. A., Mehmood S., Yasar M., Naeem A., Bhatti A. S. (2014) Effect of varied ag nanoparticles functionalized cnts on its anti-bacterial activity against e. coli. *Sens. Actuator A-Phys.* 216:287–294.
- Khani P. H., Zand A. N. M., Imani S., Rezayi M., Rezaei-Zarchi S. (2011) Determining the antibacterial effect of zno nanoparticle against the pathogenic bacterium, shigella dysenteriae (type 1). *International Journal of Nano Dimension.* 1 (4): 279–285.

- Khodkar A., Khezri S. M., Pendashteh A. R., Khoramnejadian S., Mamani L. (2019) A designed experimental approach for photocatalytic degradation of paraquat using α -Fe₂O₃@mil-101(cr)@TiO₂ based on metal-organic framework. *Int. J. Environ. Sci. Technol.* 16:5741–5756.
- Khoramnejadian S. (2011) Kinetic study of biodegradation of linear low density polyethylene/chitosan. *Adv. Environ. Biol.* 5 (10): 3050–3055.
- Khoramnejadian S., Fatemi F. (2016) Assessment of microbiological quality of the river damavand in Iran by measuring coliform bacteria, nitrate and pH of water in autumn. *J. Biol. Today's World.* 5 (4): 76–80.
- Kim J. D., Yun H., Kim G. C., Lee C. W., Choi H. C. (2013) Antibacterial activity and reusability of CNT-AG and GO-AG nanocomposites. *Appl. Surf. Sci.* 283:227–233.
- Kot M., Kalinska A., Jaworski S., Wierzbicki M., Smulski S., Golebiewski M.A. (2023) In vitro studies of nanoparticles as a potentially new antimicrobial agent for the prevention and treatment of lameness and digital dermatitis in cattle. *Int J Mol Sci.* 24 (7): 6146.
- Li Z., Fan L., Zhang T., Li K. (2011) Facile synthesis of Ag nanoparticles supported on MWNTs with favorable stability and their bactericidal properties. *J. Hazard. Mater.* 187 (1-3): 466–472.
- Liu H., Li H., Cheng W. J., Yang Y., Zhu M. Y., Zhou C. R. (2006) Novel injectable calcium phosphate/chitosan composites for bone substitute materials. *Acta Biomater.* 2:557–565.
- Lloyd D. R., Phillips D. H. (1999) Oxidative DNA damage mediated by copper (II), iron (II) and nickel (II) Fenton reactions: evidence for site-specific mechanisms in the formation of double-strand breaks, 8-hydroxydeoxyguanosine and putative intrastrand cross-links. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 424 (1-2): 23–36.
- Marambio-Jones C., Hoek E. M. V. (2010) A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *J. Nanoparticle Res.* 12 (5): 1531–1551.
- Meghana S., Kabra P., Chakraborty S., Padmavathy N. (2015) Understanding the pathway of antibacterial activity of copper oxide nanoparticles. *RSC Adv.* 5:12293–12299. <https://doi.org/10.1039/C4RA12163E>
- Moghanjooghi S. Mousavi, Khoramnejadian S., Fataei E., Monsan A. A. (2022) Laboratory investigation of arsenic removal from the aquatic environment using nano adsorbents extracted from native zeolite. *Main Group Chem.* 21 (1): 113–123.
- Mollania N., Gharibnia F., Dizaj R., Rostami-Taghi, Kheyraadi M. (2016) Study on the antibacterial effects of silver nanoparticles produced by α -amylase enzyme. *J. Sabzevar Univ Med Sci.* 23 (2): 214–221.
- Nasehnia F., Ganjipour B., Yousefnejad E., Mohajerzadeh S., Miri A. M., Arzi E. (2004) Fabrication and study of carbon nanotube by plasma enhanced chemical vapor deposition (PECVD). *Iran. J. Phys. Res.* 4 (2): 103–108.
- Nohegar S., Ashraf, Nejaei A., Fataei E., Ramazani M. E., Eslami P., Alizadeh (2022) Evaluation of the toxicity of zinc oxide nanoparticles upon Staphylococcus aureus and Escherichia coli in contaminated water. *J Adv Environ Health Res.* 10 (3): 235–246.
- Ohadian A., BKhayat N., Mokhberi M. (2023) Long-term and microstructural studies of soft clay stabilization using municipal solid waste and nano-MGO as an eco-friendly method. *Anthropog. pollut.* 7 (1): 43–54.
- Orhan I. E., Ozcelik B. E. R. R. I. N., Sener B. (2021) Evaluation of antibacterial, antifungal, antiviral, and antioxidant potentials of some edible oils and their fatty acid profiles. *Turkish Journal of Biology* 35 (2): 251–258.
- Osouledini N., Mahlooji A. H., Abdollahzadeh M. (2024) Investigating the effect of pulsed electric field (PEF) on Escherichia coli (E. coli) bacteria as an indicator of water contamination. *Anthropog. pollut.* 7 (2): 1–11. <https://doi.org/10.57647/j.jap.2023.0702.22>
- Pereyra I., Alayo M. I. (1997) High quality low temperature PECVD silicon dioxide. *J. Non-Cryst. Solids.* 212 (2-3): 225–231.
- Prodana M., Ionita D., Ungureanu C., Bojin D., Demetrescu I. (2011) Enhancing antibacterial effect of multiwalled carbon nanotubes using silver nanoparticles. *Digest J. Nanomater. Biostruct.* 6 (2): 549–556.
- Raffi M., Mehrwan S., Bhatti T. M., Akhter J. I., Hameed A., Yawar W., Hasan M. M. (2010) Investigations into the antibacterial behavior of copper nanoparticles against Escherichia coli. *Ann. Microbiol.* 60:75–80.
- Saberinia F., Farhangi M. B., Mahabadi N., Yaghmaeian, Ghorbanzadeh N. (2021) Investigation of Gowharrood.
- Saleemi M. A., Yahaya N., Zain N. N. M., Raouf M., Yong Y. K., Noor N. S., Lim V. (2022) Antimicrobial and cytotoxic effects of cannabinoids: an updated review with future perspectives and current challenges. *Pharmaceuticals* 15 (10): 1228.
- Samarajeewa A., Velicogna J., Princz J., Subasinghe R., Scroggins R., Beaudette L. (2017) Effect of silver nanoparticles on soil microbial growth, activity and community diversity in a sandy loam soil. *Environ Pollut.* 220:504–513.
- Semisch A., Ohle J., Witt B., Hartwig A. (2014) Cytotoxicity and genotoxicity of nano- and microparticulate copper oxide: role of solubility and intracellular bioavailability. *Particle and fibre toxicology* 11:1–16.

- Seo Y., Hwang J., Kim J., Jeong Y., Hwang M. P., Choi J. (2014) Antibacterial activity and cytotoxicity of multi-walled carbon nanotubes decorated with silver nanoparticles. *Int J Nanomedicine*. 9:4621–4629. <https://doi.org/10.2147/IJN.S69561>
- Shanthi R., Poornima D., Naveen M., Thangaradjou T., Choudhury S. B., Rao K. H., Dadhwal V. K. (2016) Air-sea CO₂ flux pattern along the southern bay of Bengal waters. *Dynamics of Atmospheres and Oceans* 76:14–28.
- Shvedova A. A., Pietroiusti A., Fadeel B., Kagan V. E. (2012) Mechanisms of carbon nanotube-induced toxicity: focus on oxidative stress. *Toxicol. Appl. Pharmacol.* 261 (2): 121–133.
- Sotto A. Di, Chiaretti M., Carru G. A., Bellucci S., Mazzanti G. (2009) Multi-walled carbon nanotubes: lack of mutagenic activity in the bacterial reverse mutation assay. *Toxicol. Lett.* 184:192–197.
- Stoimenov P. K., Zaikovski V., Klabunde K. J. (2003) Novel halogen and interhalogen adducts of nanoscale magnesium oxide. *Journal of the American Chemical Society* 125 (42): 12907–12913.
- Tarlani A., Fallah M., Lotfi B., Khazraei A., Golsanamlou S., Muzart J., Mirza-Aghayan M. (2015) New ZnO nanostructures as non-enzymatic glucose biosensors. *Biosensors and Bioelectronics* 67:601–607.
- Tehrani M. R. Fadaei, Skandari S. (2023) Removal of organic pollutants from water by improved carbon nanotubes. *Journal of Water and Wastewater; Ab va Fazi-lab* 34 (4): 1–21. <https://doi.org/10.22093/wwj.2023.385457.3324>
- Yun H., Kim J. D., Choi H. C., Lee C. W. (2013) Antibacterial activity of CNT-AG and GO-AG nanocomposites against gram-negative and gram-positive bacteria. *Bull. Korean Chem. Soc.* 34 (11): 3261–3264.
- Zainalzadeh D., Kalantari R. Rezae, Nodehi R. Nabih Zadeh, Esrrailly A. (2015) Production and testing of magnetic nanoparticles (Fe₃O₄-AG) for disinfection of urban wastewater. *16th National Conference on Environmental Health*.
- Zhang D., Ma X. L., Gu Y., Huang H., Zhang G. W. (2020) Retracted: green synthesis of metallic nanoparticles and their potential applications to treat cancer. *Frontiers in Chemistry*. 8:799.
- Zhang W., Zhang Y. H., Ji J. H., Zhao J., Yan Q., Chu P. K. (2006) Antimicrobial properties of copper plasma-modified polyethylene. *Polymer*. 47 (21): 7441–7445. <https://doi.org/10.1016/j.polymer.2006.08.057>