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Wastewater Disinfection by Synthesized Copper Oxide Nanoparticles Stabilized with Surfactant

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This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master of in Chemistry, Faculty of Graduate Studies, An-Najah National University, Nablus, Palestine.
2013

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Dedication

To my honorable father and affectionate mother.

To all my beloved family; Fadi, Mohammed, Hussein, Amr, Fida', Mohammed and Haneen.

To my grandparents, uncles, aunts, cousins and all my relatives.

To all those stay in my mind and heart.

To all my friends in my dear village Immatain and in everywhere, especially lovely friends; Kamal Barri, Alaa' Metani, Rami A. Suwan, Diaa Aref and Anas Al-Ali.

To all above, I'd like to dedicate this work on behalf of them, and I ask ALLAH to consider this action as right deeds and accept it from me

Best Regards

Muath Mousa

Acknowledgment

For everything and anything my deep thankful to Al-Majid ALLAH.

I would like to express my deepest appreciation to my advisors Dr. Mohammad Suleiman and Dr. Amjad Hussien for their endless directions and their help to complete this thesis even in their rest time.

I would like to thank the technical staff in Chemistry and Biology Laboratory Sciences Departments, for their technical help, especially, Mr Nafez Dweikat and Miss Lina Dawabsha.

I would also like to thank technical staff in Poison and Chemical and Biological Analysis Centreat Najah University, especially, Dr Amjad Hussein, Asma' and Samah. For assistance in chemical and biological analysis.

I would like to thank technical staff in King Abdullah Institute for Nanotechnology (KAIN) at king saud university, for their help in XRD and SEM measurements.

I would like to thank Nablus municipality employers especially water department for the providing of all needed wastewater samples.

Special thanks to my cousin Yousef Barri for his great help in providing all needed articles.

Finally endless thanks for my father, mother, brothers and my uncles Mousa, Ahmad Barri and Ammar Ghanem for their continuous encouragement, supporting and their patience during the study period including the difficult days of detentions.

الإقرار

أنا الموقع أدناه موقع الرسالة التي تحمل العنوان:

Wastewater Disinfection by Synthesized Copper Oxide Nanoparticles Stabilized with Surfactant

أقر بأن ما اشتملت عليه الرسالة هو نتاج جهدي الخاص، باستثناء ما تمت الاشارة اليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provide in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالب:**

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List of Abbreviations

Symbol	Abbreviation
CuO-TOAB(1)	Copper oxide nanoparticles stabilized with TOAB surfactant prepared at 65 ºC
CuO-TOAB(3)	Copper oxide nanoparticles stabilized with TOAB surfactant prepared at 75 ºC
CuO-TOAB(5)	Copper oxide nanoparticles stabilized with TOAB surfactant prepared at 85 ºC
CuO(2)	Copper oxide nanoparticles without TOAB surfactant prepared at 65 ºC
CuO(4)	Copper oxide nanoparticles without TOAB surfactant prepared at 75 ºC
CuO(6)	Copper oxide nanoparticles without TOAB surfactant prepared at 85 ºC
XRD	X-Ray Diffraction
SEM	Scanning electron microscope
TDS	Total dissolved solids
MCL	Maximum contaminant level
TSS	Total suspended solids
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
E. coli	*Escherichia coli*
TC	Total coliforms
FC	Fecal coliforms
E. faecalis	*Enterococcus faecalis*
DBPs	Disinfectant byproducts
WHO	World Health Organization
CFU	Colony-forming *unit*
MBC	Minimum bactericidal concentrations
ROS	Reactive oxygen species
QACs	Quaternary ammonium compounds
TOAB	Tetraoctylammonium bromide
NPs	Nanoparticles

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Abstract

CuO NPs were prepared by a quick precipitation method in the absence and presence of tetraoctylammonium bromide (TOAB) that was used as a stabilizer to control the nanoparticles size. X-Ray Diffraction (XRD) and Scanning electron microscope (SEM) were used to characterize CuO NPs. NPs average size was from 7-12 nm with rod-like shape that was controlled by the change of preparation temperatures and the presence of TOAB surfactant.

The antibacterial activity of the prepared CuO NPs were evaluated using total coliform (TC), fecal coliform (FC) and *Enterococcus faecalis* (*E. faecalis*) bacteria counts in wastewater. Different parameters were studied to obtain the optimum wastewater disinfection conditions, these parameters are size of nanoparticles with and without TOAB surfactant, nanoparticles concentration, contact time, pH, shaking and temperature of wastewater.

CuO-TOAB stabilized NPs showed higher antibacterial activity more than that without TOAB surfactant, where it was less than 100 and 1000 µg/ml for CuO NPs with and without TOAB surfactant, respectively. The effect of NPs size were studied where the sizes for CuO-TOAB stabilized NPs was found to be

11.5, 9.9 and 7.8 nm, while the sizes of CuO non-stabilized NPs was found to be 12.4, 11.4 and 9.1 nm, however, medium size for both CuO-TOAB stabilized NPs (9.9 nm) and CuO non-stabilized NPs (11.4 nm) have the highest antibacterial activity of other sizes. Contact time effect was small as there was slight difference in the antibacterial activity of both CuO NPs with and without TOAB. Noticeable high activity of CuO NPs with and without TOAB surfactant occurred when wastewater samples were treated at 25 ºC and 35 ºC, respectively. The antibacterial activity of CuO NPs with and without TOAB surfactant slightly increased by decreasing wastewater pH values. Antibacterial activity of CuO NPs without shaking showed lower activity of about 70 and 90% for CuO NPs without and with TOAB surfactant in comparison to the antibacterial activity with shaking. In all parameters were studied, the antibacterial activity of both CuO NPs with and without TOAB surfactant were higher against gram positive bacteria (*E. faecalis*) compared to the activity against gram negative (TC and FC). Flow up test proved the applicability of CuO-TOAB NPs as a novel wastewater disinfection technique.

Chapter 1

Introduction

1.1 Wastewater

1.1.1 Wastewater overview

Wastewater is a waste liquid product of municipal, industrial and agricultural activities that contains pollutants such as microorganisms, organic materials, soluble inorganic compounds and toxic heavy metals. These pollutants change the physical, chemical and biological characteristics of clean water [1, 2]. It can be classified according to the waste sources into municipal and industrial wastewater, where the municipal waste sources are homes and commercial activities; that often contain feces and urine, but the sources of industrial wastewater are the industrial and agricultural activities; that contain in addition to the domestic compositions; organic and inorganic chemicals [2, 3]. Wastewater contains high concentrations of microorganisms such as viruses, bacteria, and protozoa, and toxic chemicals such as trace elements, heavy metals and radionuclides. Therefore wastewater is one of the most important sources to waterborne diseases, some of which are fatal such as typhoid and cholera. The polluted water was the cause of death of about 1.6 million people under 5 year age in 2004 [4-6].

Wastewater treatment is one of the most important issues nowadays according to the toxic effects of wastewater pathogenic and hazardous pollutants on human, agriculture and animals. To protect the environmental from pollution,

wastewater treatment must be considered on personal and governmental level investigation. Wastewater treatment may involve physical, chemical and biological processes to clean up water from different contaminants [7, 8]. Wastewater treatment is elucidated in more detail throughout this chapter.

1.1.2 Wastewater characteristics

The wastewater characteristics can be classified into physical, chemical and biological characteristics.

1.1.2.1 Physical characteristics

There are many physical characteristics of wastewater such as total (Fixed, volatile, dissolved and suspended) solids, color, odor, and others [9]. However, total dissolved solids (TDS) are the dissolved matters in wastewater, where these matters can be inorganic salts and metals such as bicarbonates, chlorides, calcium, magnesium, potassium and sodium including small amounts of organic materials. These particles sizes for dissolved solids ranged from 0.01 to 1.00 µm [10, 11].

According to United States environmental protection agency (USEPA), the maximum contaminant level (MCL) of TDS in drinking water must be less than 500 ppm, because high levels of TDS can cause many effects such as dry skin and get poor taste [12]. Total suspended solids (TSS) are the suspended inorganic and organic materials in wastewater, TSS retained in a 1.2 µm pore size filter when wastewater filtered, where dissolved solids passed through the same filter [13].

1.1.2.2 Chemical characteristics

The chemical pollutants in wastewater can be classified into organic, inorganic and gaseous chemicals. These pollutants are summarized in table 1.1 [2].

Table 1.1 organic, inorganic, gases wastewater pollutants[2]

Organic	Inorganic
Carbohydrates	Chlorides
Fats, oils, and grease	Heavy metals
Pesticides	Nitrogen
Proteins	Phosphorus
Phenols	Priority pollutants
Surfactants	Sulfur
Gases	
Hydrogen sulfide	Methane

1.1.2.2.1 Organic pollutants

Organic pollutants in wastewater in general consist of proteins, carbohydrates and fats and oils; at approximately 50, 40 and 10 %, respectively. Priority pollutants, surfactants, and emerging contaminants represent trace organic pollutants in wastewater [2]. Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) considered as the most practical indicators of water organic pollutants quality.

BOD is defined as the dissolved oxygen in wastewater required for aerobic microorganisms that breakdown the organic pollutions as shown in equation (1.1), so it used as a parameter to determine the concentrations of organic matters in wastewater. The most widely used test is $(BOD)_5$ where it measures the BOD of wastewater during 5 days at 20º C [5, 2].

$$\text{Organic matter} + \text{nutrient} + O_2 \xrightarrow{\text{biomas}} CO_2 + H_2O + \text{biomass} \qquad \text{eq(1.1)}$$

Chemical Oxygen Demand (COD) is defined as the consumed dissolved oxygen in wastewater by oxidizing all organic pollutants using strong chemical oxidizing agent like potassium dichromate, so COD must be higher than BOD because the compounds oxidized chemically is more than compounds oxidized biologically [15, 16].

1.1.2.2.2 Inorganic pollutants

Wastewater contains many inorganic pollutants such as heavy metals, nitrogen compounds, phosphorus trace elements and other toxic inorganic constituent. Some inorganic pollutants are listed in Table 1.1. Toxic inorganic metals such as Arsenic (As), Barium (Ba), Cadmium (Cd), Lead (Pb), Mercury (Hg) and others may exist in drinking water in limited quantities according to its health effects.

Cadmium (Cd) usually has +2 oxidation state but also has +1 state, it must be less than 1 µg/L. Food or water containing cadmium cause health effect on the animal and human like high blood-pressures, liver disease, nerve or brain damage, painful osteomalacia (bone disease) and kidney damage [5, 17, 18].

Lead (Pb) the main sources of lead in water are manufacturers, mining, plumbing, and deposition of gasoline exhausts [19]. High level of lead more

than (0.015 mg/L) in water causes some health effects such as central and peripheral nervous system effects and kidney damage [20, 21].

Mercury (Hg) is used in many applications such as in thermometers, antiseptics, batteries and dental amalgams. It can be found in water in the form of organometallic compounds (such as alkylmercurials), inorganic compounds (such as $HgCl_2$) and elemental mercury. There are many health effects of mercury such as kidney damage; therefore, the MCL for Hg in drinking water is very low about 0.002 mg/L [22, 23].

Other toxic elements exist in water with its effective and MCL (mg/L) according to EPA for drinking water quality are listed in table 1.2 [24].

Table 1.2 Metals that contaminate the water, their health effects and their MCL (mg/L) according to EPA for drinking water quality [24].

Metal contaminant	Health effects	MCL (mg/L)
Arsenic	Nervous system effects	0.05
Asbestos	Possibly carcinogenic	7 Million fibers per liter (MFP)
Barium	Circulatory system effects	2
Cadmium	Kidney effects	0.005
Chromium	Liver, kidney, digestive system effects	0.1
Copper	Digestive system effects	1.3
Mercury	Kidney, nervous system effects	0.002
Nickel	Heart, liver effects	0.1
Nitrate	Blue-baby effect	10

Water quality can be affected by other inorganic pollutants such as: pH, free and total chlorine, sulfate, phosphorus, sodium and nitrogen. However; the optimum pH for drinking water should be less than 8, also to keep on the drinking water quality the concentrations of sodium and sulfate should be less than 200 and 250

mg/L; respectively. However; the presence of sulfate at high concentrations can causes noticeable taste and laxative effect, also higher concentrations of chlorine more than 5 mg/L cause taste or smell for water [10].

1.1.2.3 Biological characteristics

In addition to chemical and physical characteristics of wastewater, there are biological characteristics. The biological pollutants are living pathogenic microorganisms that exist in wastewater. The main wastewater microorganisms are bacteria, viruses and protozoa that can cause acute and chronic health effects.

Bacteria are single celled organisms classified as prokaryotic organisms [14]. Bacteria have different shapes such as spheres, rods and spirals; for *Streptococcus*, *Bacillus subtilis* (*B. subtilis*) *and Vibrio cholera*, respectively [25]. The size of bacteria can vary according to kind and shape but in general the sizes range from 0.1 to 2 µm [26].

The different kinds of bacteria in wastewater can cause different waterborne diseases such as *cholera*, *typhoid* and *shigella*. However; many types of bacteria can exist in wastewater with less serious manifestations such as *Escherichia coli* (*E. coli*), *Enterobacter*, *Klebsiella pneumonae*, *Streptococcus faecalis* (*S. faecalis*) and others. Various bacteria kinds with its diseases are listed in table 1.3 [27, 28].

Table 1.3 Various bacteria kinds with their health effects. [28]

Bacterium/Bacteria	Disease
Actinomyces israelii	Actinomycosis
Camplyobacter jejuni	Gastroenteritis
Clostridium perfringens	Gangrene (gas gangrene)
Clostridium tetani	Tetanus
Escherichia coli—enteroinvasive	Gastroenteritis
Escherichia coli—enteropathogenic	Gastroenteritis
Escherichia coli—enterotoxigenic	Gastroenteritis
Escherichia coli—enterohemorrhagic 0157:H7	Gastroenteritis and hemolytic uremic syndrome
Leptospira interrogans	Leptospirosis
Mycobacterium tuberculosis	Tuberculosis
Nocardia spp.	Nocardosis
Salmonella paratyphi	Paratyphoid fever
Salmonella spp.	Salmonellosis
Salmonella typhi	Typhoid fever
Shigella spp.	Shigellosis
Vibrio cholerae	Cholera (Asiatic chlorea)
Vibrio parahaemolyticus	Gastroenteritis
Yersinia enterocolitica	Yersiniosis (bloody diarrhea)

Total coliforms (TC), fecal coliforms (FC), and fecal streptococci can be used as indicators for the presence of microorganisms in wastewater [29].

Total Coliforms (TC) is the group of total coliforms that belong to gram-negative bacteria, nonspore-forming, where their shape is rod-shaped, the optimal temperature of ferment lactose with gas production at 35 °C. There are many bacteria kinds belong to total coliforms such as *E. coli*, *Enterobacter, Klebsiella, and Citrobacter*. These bacteria are found in high numbers in human and animal feces so the total coliform is used as indicator to feces pollution of wastewater and drinking water. Generally the total coliform test is used as a surrogate to *E. coli* test [29, 27].

Fecal Coliforms (FC) in wastewater and drinking water indicate the presence of warm-blooded fecal pollution. The group of fecal coliforms includes all

coliforms that have ability to produce colony at an elevated incubation temperature at 44.5 ºC for 24 h such as *E.coli* and *Klebsiella pneumonae* [27, 29].

Fecal Streptococci (*Enterococcus faecalis* (*E. faecalis*)) indicate the presence of human and warm-blooded animals fecal in water. Fecal Streptococci in wastewater usually less than the presence of total and fecal coliforms. Fecal Streptococci also known Enterococci include *S. faecalis, S. faecium* and others [29, 30, 27].

1.1.3 Wastewater treatment

Due to increasing demands on clean water for many purposes such as drinking, industrial and irrigation purposes, with the shortage of clean water sources because of population growth, increasing industrial demands and other reasons. However; to decrease the health effects of pathogens and hazard chemicals found in wastewater, nowadays wastewater treatment is one of the most priorities issues facing the world to meet clean water demands [31, 32].

Wastewater treatment is a multi-stage process including chemical, physical and biological processes on wastewater to clean it from pathogens, hazards chemicals, solids and other water contaminants. These processes improve the water quality for irrigation, industrial and other beneficial purposes [33, 34].

There are many physical, chemical and biological wastewater treatment techniques. Physical methods such as screening, sedimentation and filtration, however; biological processes as biological trickling filters, finally chemical

techniques such as coagulation, ion exchange, precipitation and chemical disinfection [35].

1.1.3.1 Wastewater disinfection

Disinfection is a process that aims to destroy the pathogenic microorganisms in wastewater by many chemical and physical methods such as chlorination, chloramines, ozone, and nanofiltration. Some of disinfectants with its advantage and disadvantages are listed in table 1.4 [36].

Table 1.4 Advantages and disadvantages of some most widely used disinfectants [36]

Disinfectant	Advantages	Disadvantages
Free chlorine (NaOCl, $Ca(OCl)_2$)	Easy to use ; effective against most pathogens; stable residual	Not available worldwide; some users object to taste and odor
Electro-chemically genderated oxidant from NaCl	Easy to use; effective against most pathogens; stable residual	Not available worldwide; some users object to taste and odor mostly chlorine
Chloramines (Monochloramine)	Stable residual	Less effective Microbiocide than free chlorine; requires skill and equipment to generate on-site; household use impractical
Ozon	Highly micro-biocidal	No residual; generate onsite; hard to use; need special facelities and trained personnel; hazardous
Chlorine Dioxide	Highly micro-biocidal	Poor residual; generate onsite; some technologies require special facelities , trained personnel and are hazardous toxicologic concerns

Acids (especially) lime juice and mineral acids) and hydroxide (caustic)	Acids inactivate v.cholerae & some other bacteria; limes and chemicals widely available	Limited microbiocidal activity; CaO use requires special facilites and trained personnel and is hazadous; CaO process difficalt to control
Combined chlorination, coagulation-flocculation-filtration systems	Highly effective for microbe reductions	Availability now limited; requires some training and skill; efficacy varies with water quality

The most widely used disinfectant is free chlorine (chlorine gas, sodium hypochlorite and solid calcium hypochlorite) according to its high effeciency against bacteria killing more than 99% of microbes and its low cost [37,36]. When chlorine react with the organic, ammonia, and phenolic matter in water there are undesirable disinfectant byproducts (DBPs) produced such as trihalomethanes (THMs), haloacetic acids (HAAs), and haloacetonitriles [38, 39]. These byproducts are considered as carcinogenic and harmful for the environment even at low concentrations [38]. Other disadvantages of chlorine gas are the difficulty of transport and storage where it can be stored as a liquefied gas under high pressure [36]. Sodium hypochlorite and solid calcium hypochlorite are more expensive than chorine gas [41].

Chlorine dioxide disinfectant has less DBPs, ten times higher solubility in water than free chlorine [42], and is not affected by pH where chlorine disinfectant is less effective at high pH. Chlorine dioxide also has many disadvantages such as decomposition of chlorine dioxide under sunlight, and

because chlorine dioxide gas is explosive under pressure, the disinfection process should be made in situ [41].

Other common disinfectant is monochloramine (NH_2Cl) where it is less effective and requires long contact time for inactivation of bacteria in comparison with other disinfectants, its efficiency decreases with increasing pH and it must be used in situ. This disinfectant has many advantages such as: it has less hazardous DPBs, its residual is more stable than other disinfectants, it is inexpensive and easy to make [41, 43].

One of the strongest disinfectants is ozone. It has many advantages such as: it has higher efficiency (to remove some pathogens such as viruses) than chlorine, chloramines, and chlorine dioxide. It needs very short contact time with pathogens to kill them. No noticeable DBPs in the absence of bromide, its activity is not affected by pH. However, it is still not recommended for water treatment because ozone is hazardous and toxic gas, so process should be conducted carefully in the presence of bromide in water as DBPs are formed, it requires expensive specialized equipment, requires high maintenance and operator skill levels, and should be generated on-site using high electricity [36, 41].

Although there is a tremendous development of water disinfection processes, the world still facing water quality issues, especially in the waterborne diseases as one of the major global problems according to World Health Organization (WHO). Moreover, the by-products, the cost of disinfection

methods and other environmental disadvantages encouraged the search for new disinfection technology to reduce the conventional disinfectants disadvantages [34, 44]. Nowadays, a lot of water disinfection research focus on using nanotechnology such as nanoparticles and nano-membranes, as it has high reactivity due to its high surface to volume ratios [32, 52].

1.2 Introduction to nanotechnology

Nanomaterials, like nanoparticles, nanotubes, nanowires and thin films, are defined as very small aggregate of atoms with less than 100 nm dimension [45]. The importance of nanoparticles is due to the unique different physical, chemical and biological characteristics compared to the bulk scale, due to their high surface-to-volume ratio [46]. There are various applications for the nanotechnology such as fuel cells, hydrogen storage antibacterial activity and water treatment [47].

1.2.1 Nanoparticles preparation methods:

Metal nanoparticles can be prepared by chemical methods such as chemical reduction and electrochemical techniques [48], and physical methods such as condensation and laser ablation [46]. To prevent undesired aggregation of the nanoparticles during nanoparticles synthesis processes, stabilizers should be used, such as polymer, solid matrix and surfactants. Surfactants have many activities in chemical and biological applications and can influence the efficiency of the nanoparticles in various applications [49].

Electrochemical technique is one of the chemical nanoparticles preparation methods, this method can be summarized into six steps. Oxidative dissolution of anode by applying current density on it, migration of these dissolved ions to the cathodes, ion reduction at the cathode to zero-valent state, aggregation to form particles by nucleation and growth, arrest of growth by stabilizers to prevent the nanoparticles from non-desired aggregation, and finally precipitation of the produced nanoparticles. The size of the nanoparticles can be simply controlled by varying of temperature, current density, solvent and concentrations of reducing agent and ions [50].

Simple, inexpensive and quick chemical methods for nanoparticles synthesis is salt reduction method. This is the most common process for nanoparticles synthesis, where metal salts dissolve in a solvent such as alcohol or water, salts then dissociate to metal cations and nonmetal anion. By adding reducing agent, the metal cations are reduced to zero-valent metal aggregate to form particles by nucleation and growth. The particles are stabilized by adding stabilizing agent like citrate to prevent the nanoparticles from non-desired aggregation, then the final nanoparticles form precipitate. Size of the nanoparticles can be controlled by varying of pH, temperature, solvent type and reducing agent type [51, 46].

1.2.2 Wastewater disinfection using nanoparticles

One of the most important application for nanoparticles is water purification from chemical toxic contaminants and pathogenic. However, different

nanoparticles are used to wastewater disinfection such as silver NPs, titanium dioxide NPs and zinc oxide NPs [52].

1.2.2.1 Silver nanoparticles as disinfectant

The most nanoparticles used as water disinfectant is silver nanoparticles due to its low toxicity on human cells and high efficiency against microorganisms. Sondi *et al*. reported that silver nanoparticles have high activity against *Escherichia coli* (*E. coli*) as a model for Gram-negative bacteria [53].

The antimicrobial activity can be affected by the particle size and surfactants that used in the particles synthesis, the smaller particles size have higher bactericidal than larger ones [48]. Many surfactants can be used in silver nanoparticles synthesis where Libor Kvı´tek et al used sodium dodecyl sulfate-SDS and polyoxyethylenesorbitane monooleate-Tween 80 as surfactant and polyvinylpyrrolidone- PVP 360 as a polymer stabilizer, where this study revealed that the surfactants enhanced Ag NPs antimicrobial activity [54]. Other variable can affect the bacteria activity is the pH changes where the antibacterial activity increases with decreasing of pH [55].

1.2.2.2 TiO_2 nanoparticles as disinfectant

TiO_2 are a stable and harmless semiconductor photocatalyst particles, its oxidation and reduction reactions activated by solar spectrum about $\lambda < 385$ nm, TiO_2 nanoparticles nowadays is widely used as air and water disinfectant [56].

Saeed Rezaei-Zarchi, *et al.* studied the *E. coli* inactivation by TiO_2 nanoparticles [57]. Other antibacterial activity studies of TiO_2 was by Fu *et al.*

where investigate the *E. coli* as a Gram-negative bacterium and *B. megaterium* as Gram-positive bacterium. Good inhibition effect for *E. coli* suspended using gold-capped TiO_2 nanoparticles was reported [58].

1.2.2.3 ZnO nanoparticles as disinfectant

ZnO is semiconductor can be excited by small amount of UV. Dissolution behavior is an important property of ZnO nanoparticles and considered as important nanomaterials disinfectant for a Gram-negative bacterium and Gram-positive bacterium [59].

Adams et al. reported that ZnO nanoparticles has antibacterial activity on *B. subtilis* and *E. coli*, the study reported that antibacterial activity does not affected by different particles sizes and its activity is the same under dark and light activity on *B. subtilis* bacteria but more activity under light on *E. coli* [60]. Li *et al*. reported that the toxicity of ZnO nanoparticles on *E. coli* increases with decreasing pH [56]. Premanathan *et al*. reported that the bacterial activity of ZnO NPs on Gram-positive bacterium such as *S. aureus* is more effective than on Gram-negative bacteria such as *E. coli* [62]. Alaa *et al.* reported that the bacterial activity of ZnO nanoparticles that stabilized with tetraoctylammonium bromide (TOAB) surfactant is higher than that without surfactant against *E. coli, S. aureus* and *B. subtilis* [98]. Sondos *et al*. reported that ZnO nanoparticles showed good antibacterial activity against *E. coli* under solar light, while the antibacterial activity of ZnO nanoparticles increased when using anthocyanin dye as sensitizer for ZnO [99].

1.3 Copper oxide nanoparticles preparation and its antibacterial activity

1.3.1 History of copper and its antibacterial activity

The exact time of discovery copper is not known but it is estimated around 9000 B.C in the Middle East [63]. Copper is the most ancient metal used, where it was used by Egyptian around 2000 BC as a sterilize of wounds and water, Copper has a lot of properties such as good corrosion resistance, low-cost and antimicrobial activity [64, 65].

One of the most important applications of copper and copper compounds is bacteria disinfectant, due its versatility, low cost, essential for humans at low levels and biocide activity properties [64, 66]. *V.B.P.* Sudha *et al.* test the effect of copper device (15.2 cm^2/L surface area of copper coil to volume of water) on *E. coli*, *S. typhi* and *V. cholerae* in water and the result shows that when the device was on glass bottle there is no growth of *E. coli*, *S. typhi* and *V. cholerae* after overnight incubation where it was 935, 688 and 502 CFU, respectively, before incubation [67]. Gustavo Faúndez *et al.* studied the activity of copper against suspension *Salmonella enterica* and *Campylobacter jejuni* at different temperatures; 10 and 25 ºC,the result showed that copper surface has good antibacterial activity at these temperatures but more efficiency at 25 °C [68].

1.3.2 Methods forpreparation of copper oxide nanoparticles and its antibacterial activity

Copper(II) oxide is semiconducting compound that belongs to monoclinic structure systems. It has many useful physical and chemical properties such as superconductivity at high temperature, photovoltaic properties, relatively stable, low cost and has antimicrobial activity [69]. CuO nanoparticles also have various technology applications such as catalysis [70], batteries due to high electrochemical capacity [71], and gas sensors [72]. Varying sizes and shapes can be synthesized for CuO nanoparticles by different methods such as sonochemical technique [73], electrochemical method [74], high temperature combustion [75] and novel quick precipitation method [76].

CuO nanoparticles synthesis by novel quick precipitation (salt reduction) method is very interesting because it is safe, simple, environmentally friendly method and gives large scale of nanoparticles [77, 78]. Zhu *et al*. prepared highly dispersed CuO nanoparticles using copper acetate aqueous solution as a precursor and sodium hudroxide (NaOH) as reducing agent. The average size of CuO NPs product was 6 nm [76]. Wu *et al*. prepared well dispersed CuO nanoparticles by dissolving $Cu(NO_3)_2 \cdot 3H_2O$ in N,N-Dimethylacet amide (DMAC) and using NaOH solid as reducing agent, different size of CuO NPs were obtained by using NaOH at different temperatures [77].

Fathima *et al.* prepared stabilized CuO nanorods with sodium dodecylsulfate (SDS), dodecyltrimethylammonium bromide (CTAB) and Triton X-100 as

anionic, cationic and neutral surfactants, respectively, using fast and simple quick precipitation method. The ionic SDS surfactant has the strongest interaction with the cationic CuO NPs according to negative charge of SDS surfactant. However; other results showed that the surfactants play important roles in the shape and applications of the nanomaterials [79].

Researches on the antimicrobial activity of CuO NPs are limited. Ren *et al.* reported that CuO NPs has antibacterial activity against a range of gram-positive and gram-negative bacteria such as *S. aureus*, EMRSA-15 and *E .coli* NCTC 9001 [80]. Baek *et al.* studied the antibacterial effect of CuO, NiO, ZnO, and Sb_2O_3 nanoparticles, and showed that CuO nanoparticles is the most toxic of these metal oxide nanoparticles against *E. coli* as Gram-negative bacteria and *B. subtilis* and *S. aureus* as Gram-positive bacteria. On the other hand, CuO NPs showed higher activity against *E. coli* more than against gram-positive bacteria [81]. M. Heinlaan *et al*. studied the effects of bulk and nano CuO on *Vibrio fischeri*, crustaceans Daphnia magna and Thamnocephalus platyurus. The results showed that CuO NPs have higher antibacterial activity than bulk CuO [82].

1.4 Mechanisms ofbacterial disinfection by copper nanoparticles

The exact mechanism of bacterial disinfection by CuO is not clear. However, limited proposal mechanisms are reported, one of these mechanisms is the released Cu ions from the nanoparticles come in contact with the bacteria cell membrane that damage bacteria cell membrane [80]. Ruparelia *et al.* proposed

that the released Cu ions may lead to disorder in DNA helical structure by interaction of the ions with DNA molecules [83]. Another proposed mechanism is the "nano-effect" where M. Heinlaan *et al.* reported that the size of nano copper oxide plays an important role on the toxicity and therefore, on the disinfection efficiency [82]. The latest proposed mechanism is the oxidative stress, Ivask *et al.* reported that reactive oxygen species (ROS) may be induced by CuO NPs, depending on CuO NPs dissolution rate, where ROS may cause damage to bacteria cell structure. However, the mechanism was applied to *E. coli* bacteria only [84].

1.5 Quaternary ammonium cations as disinfectant

Quaternary ammonium compounds (QACs) belong to N-containig organic cations. The general formula is R_4N^+ where N is a positive charge. Nitrogen is bonded to four carbon atoms by covalent bonds, R is a saturated or unsaturated alkyl or aryl group. Wide range of quaternary ammonium cations are produced synthetically. It has various applications such as cosmetics, asphalt emulsions and as antimicrobials [85, 86]. Tetraoctylammonium bromide (TOAB) is one of the quaternary ammonium compounds with the molecular formula $[CH_3(CH_2)_7]_4N(Br)$ and molecular weight 546.79, N. Cioffi *et al.* reported that TOAB has good antibacterial activity against *E. coli* [87].

1.6 Objectives of this study

The main objective of this study is wastewater disinfection using environmentally friendly and inexpensive copper oxide nanoparticles that

were monitored through wastewater bacterial indicators; TC, FC and *E. faecalis* counts. Further specific objectives include:

1- Size selective synthesis of copper oxide nanoparticle with and without TOAB surfactant matrix.
2- Characterization of copper oxide nanoparticles with and without TOAB surfactant using SEM and XRD to determine the nanoparticles shape and size.
3- Studying the antibacterial activity of many parameters including NPs size, stabilization, concentrations, contact time, pH, shaking and temperature of wastewater.

Chapter 2
Materials and Methods

2.1 Materials

Copper(II) sulfate pentahydrate ($CuSO_4.5H_2O$) (catalog # A11262) was purchased from Alfa Aesar, A Johnson Matthey Co., sodium hydroxide (NaOH) (catalog # 2355535200067) was purchased from Frutarom Co., sodium phosphate dibasic (Na_2HPO_4) (catalog # K28624176) was purchased from Merck Co., Citric acid (catalog # 5551100) was purchased from Frutarom Co., tetraoctylammonium bromide (TOAB) 98% purity (catalog # 294316-25G) was purchased from Sigma Co. enterococcus agar (catalog # 274620) was purchased from BD Co., violet red bile agar CM0107 purchased from Oxoid Co.

2.2 Copper oxide nanoparticles (CuO NPs) preparation

Quick precipitation method was used to prepare two types of copper oxide nanoparticles with and without tetraoctylammonium bromide (TOAB) surfactant.

The most important acronyms used in the rest of this thesis are explained in Table 2.1

Table 2.1: The most important acronyms used in the rest of this thesis.

CuO-TOAB(1)	Copper oxide nanoparticles stabilized with TOAB surfactant prepared at 65 ºC
CuO-TOAB(3)	Copper oxide nanoparticles stabilized with TOAB surfactant prepared at 75 ºC
CuO-TOAB(5)	Copper oxide nanoparticles stabilized with TOAB surfactant prepared at 85 ºC
CuO(2)	Copper oxide nanoparticles without TOAB surfactant prepared at 65 ºC
CuO(4)	Copper oxide nanoparticles without TOAB surfactant prepared at 75 ºC
CuO(6)	Copper oxide nanoparticles without TOAB surfactant prepared at 85 ºC

2.2.1 Preparation of copper oxide nanoparticles with TOAB surfactant

15.00 g of $CuSO_4.5H_2O$ with 2.34g of TOAB surfactant dissolved in 150 mL of distilled water in 250 mL round-bottom flask installed with condenser heated at about 65, 75 and 85 °C separately to produce different NPs size, after 15 min of heating and shaking at 150 rpm, 100 mL of 2M sodium hydroxide as reducing agent rapidly added to the solution, black precipitate was formed, the black precipitate was collected, washed with distilled water and then dried.

2.2.2 Preparation of copper oxide nanoparticles without surfactant

Copper oxide nanoparticles without surfactant were prepared the same as copper oxide nanoparticles with surfactant prepared steps but without presence of TOAB surfactant.

2.3 Characterization of copper oxide nanoparticles

The shape and size characterization of CuO NPs were conducted with XRD and SEM techniques

2.3.1 X-Ray diffraction (XRD) characterization

XRD technique was used to determine the structure and size of the CuO nanoparticles, using Philips-X'Pert Model-98 XRD machine with Cu source (Cu-Kα1 line, λ=1.5045 Å).

2.3.2 Scanning electron microscope (SEM)characterization

The shape and the morphology of all six CuO nanoparticles samples were characterized by SEM, the images obtained using JEOL, JSM-6360LV SEM.

2.4 Antibacterial activity

The antibacterial activity of CuO NPs were studied against total coliform (TC), fecal coliform (FC) and *Enterococcus faecalis* (*E. faecalis*) in wastewater samples. The wastewater samples were collected from western region of Nablus city sewage system by water department at Nablus municipality. The count of TC, FC and *E. faecalis* in these samples was found to be at the range of 10^3-10^4 colony forming unit CFU, and pH of 6.8.

All used tools, glassware and buffers were sterilized in an autoclave at 121 °C for 15 minutes. Autoclave were checked for working using the sterilization indicator tapes (Tiger tape).

2.4.1 Bacterial culture preparation

All used media for the bacterial indicators were prepared according to the manufacturer instructions and pouring technique were used to plate the medium as it will give more clear bacterial colonies distripution and give higher chance to count heavier plate counts.

2.4.1.1 Total coliform (TC) and fecal coliform (FC)agar preparation

Violet red bile agar was used for TC and FC count. In 1L conical flask, 19.25 g of violet red bile agar was dissolved in 500 mL distilled water; the solution was heated on heater until the solution become clear. Then tempered at 48 ºC until use.

2.4.1.2 *Enterococcus faecalis* (*E. faecalis*)agar preparation

In 1 L conical flask 21.00 g of *Enterococcus* agar was dissolved in 500 mL distilled water, the solution was heated on heater until the solution became clear. Then tempered at 48 ºC until used.

2.4.2 Plate counting method

Pour plate method was used to measure the concentrations of viable TC, FC and *E. faecalis* in wastewater samples for all treated and control samples that obtained in all following parameters studies using a sterile pipets, 1.00 mL of each sample was pipetted into the center of 3 empty petri dishes and about 20.0 mL of TC, FC and *E. faecalis*agars were poured on the samples, after that the petri dishes were rotate clockwise and anticlockwise to spread the samples throughout the agar and allowed for about 5 minutes to solidify, then the petri

dishes were inverted before incubation. The dishes that have TC and *E. faecalis* agar were incubated at 37.0 °C using Incubator (Selecta Incubator model no.0345944), while that contain FC agar were incubated at 44.5 °C for 24 h. Then the colonies in all dishes were counted using electronic colony counter (Electronic Colony Counter, catalog no. 37862-0000).

2.4.3 Wastewater disinfection using CuO NPs

Antibacterial activity of CuO NPs with and without TOAB surfactant was studied on TC, FC and *E. faecalis* in wastewater. Wastewater disinfection was studied using different parameters. The parameters were NPs size effect, the presence and absence of TOAB surfactant, nanoparticles concentrations, contact time, pH, shaking and temperature of wastewater effect.

2.4.3.1 Concentrations of CuO NPs with and without TOAB surfactant antibacterial effect

Antibacterial activities of different CuO NPs concentrations were conducted on wastewater to investigate the bacterial degradation percentage for each concentration; 1x10, $1x10^2$, $3x10^2$ and $5x10^2$ µg/mL, for CuO-TOAB(3) and $1x10^2$, $1x10^3$, $3x10^3$, $5x10^3$ and $7x10^3$, for CuO(4).

The different concentrations were obtained by preparing a stock solution of the NPs with and without TOAB in sterile distilled water were solubilization aided by sonication that were used to prepare the above mentioned final concentrations in a 10.0 mL of wastewater samples. All samples, in addition to control sample, were put in 50 mL conical flasks and then shaken for 2 h at

25 °C with 150 rpm (using Jlab tech shaker model LSB-015S purchased from Daihan labtech Co.).

2.4.3.2 NPs size and surfactant antibacterialactivity

As mentioned in NPs preparation (section 2.2), different nanoparticles size can be obtained by preparing nanoparticles at different temperature [46]. The CuO NPs with and without TOAB surfactant were prepared at 65, 75 and 85 °C.

The bacterial degradation percent of TC, FC and *E. faecalis* in wastewater sample was investigated using different size of CuO NPs with and without TOAB surfactant.

In 100 mL conical flasks about 0.010 g (10^3 µg/mL) and 0.001g (10^2 µg/mL) of each CuO nanoparticles without and with TOAB surfactant, respectively, (that prepared at 65, 75 and 85 °C), were added to 10.0 mL of wastewater samples. All samples, in addition to control sample were shaking at 150 rpm for 2 h at 25 °C.

2.4.3.3 Contact time effect

The antibacterial activity of CuO nanoparticles was tested after 0, 1, 2 and 24 h of contact time with wastewater bacteria, to study the contact time effect.

CuO-TOAB(3) and CuO(4) were used for this study. Different concentrations; 10^2 and 10^3 µg/mL of CuO-TOAB(3) and CuO(4), respectively, were obtained by weighting 0.001 g of CuO-TOAB(3) and 0.010 g CuO(4), however, control sample that used in this study was 10.0 mL of wastewater sample without any NPs addition, then, in 50 mL conical flasks, the samples were added to 10.0

mL of wastewater samples, after that, all control and NPs containing samples were shake at 25 °C with 150 rpm for 0, 1, 2 and 24 h.

2.4.3.4 Temperature of wastewater effect

The bacterial growth rate and nanoparticles activity can be affected by changing the temperature of wastewater, therefore, the antibacterial activity was studied with various temperatures 15, 25 and 35 °C

CuO-TOAB(3) and CuO(4) were used for this study. Different concentrations; 10^2 and 10^3 µg/mL of CuO-TOAB(3) and CuO(4), respectively, were obtained by weighting 0.001 g of CuO-TOAB(3) and 0.010 g CuO(4), however, control sample that used in this study was 10.0 mL of wastewater sample without any NPs addition. Then, in 50 mL conical flasks, the samples were added to 10.0 mL of wastewater samples, after that all control and NPs containing samples were shaken at 15, 25 and 35 °C with 150 rpm for 2h.

2.4.3.5 pH effect

Bacterial growth rate and CuO NPs activity can be affected by the change of wastewater pH. To investigate pH effect on the antibacterial activity of the CuO NPs, we studied the bacterial degradation percentage of CuO nanoparticles in different pH; 6.0, 7.0 and 8.0, of wastewater.

Phosphate-citrate buffer solution was used to adjust the pH values of wastewater samples. Phosphate-citrate buffer solution was prepared by mixing two stock solutions of 0.1 M (2.10 g/100 mL) citric acid and 0.2 M (2.84

g/100 mL) Na_2HPO_4. Different pH ranges from 6 to 8 were obtained by mixing the two stock solutions as in Table 2.2 [95].

Table 2.2 Different quantities of mixing citric acid solution and Na_2HPO_4 solution to obtain pH range from 6.0 to 8.0.

pH	0.1 M citric acid (mL)	0.2 M Na_2HPO_4 (mL)
6.0	3.68	6.35
7.0	1.76	8.23
8.0	0.27	9.72

CuO-TOAB(3)and CuO(4) final concentration of 10^2 and 10^3 µg/mL, respectively, were tested for pH effect at 6.0, 7.0 and 8.0. To control the pH, 1.00 mL of each maintained pH buffer solutions, listed in table 2.2, were added to each sample including the controls. The other conditions for all controls and NPs containing samples were shaking at 25 °C with 150 rpm for 2 h.

2.4.3.6 Shaking effect

Many kinds of bacteria considered as motile; that swimming in wastewater, such as most rod-shaped gram positive and negative bacteria, but other bacteria like cocci group are not motile [96]. Therefore the effect of shaking nanoparticles in wastewater on bacteria was studied.

In 50 mL conical flasks about 10.0 mL of wastewater contain 0.000g (Control), 0.001g (10^2 µg/mL) CuO-TOAB(3) NPs and 0.010 g (10^3 µg/mL) CuO(4) NPs, were shaking at 0 and 150 rpm for 2 h at 25 °C.

2.5 Flow up test

As a practical application of the prepared CuO NPs for water disinfection, flow up test were used to investigate CuO NPs antibacterial activity.

Sterile syringe column (L: 44 X D: 12 mm) was used to flow 4.00 mL of wastewater through CuO with and without TOAB surfactant layer of about 1.0 mm thickness at constant flow rate of 10 mL/min.

TC, FC and *E. faecalis* bacterial indicators were investigated before and after passing the wastewater through the CuO NPs with and without TOAB surfactant layer using the previously mentioned plate counting method.

Chapter 3

Results and Discussion

In recent years, there is an increasing concern in using nanoparticles for their antibacterial activityfor several advantages properties mainly for availability and low cost. However, CuO NPs were rarely used for their antibacterial activity due to the concerns for biological toxic effects [59, 61]. In this study, CuO NPs were prepared with and without TOAB surfactant and were investigated in a real waste water treatment samples. Different parameters that may affect the antibacterial activity were studied to obtain the optimal conditions to have NPs with high activity, low cost and low cytotoxicity to be used as wastewater disinfectant.

3.1 Synthesis and characterization of CuO NPs

CuO NPs were synthesized using quick precipitation method (eq. 3.1), in which $CuSO_4.5H_2O$ was used as Cu sources, NaOH solution and TOAB as reducing agent and stabilizer, respectively, after few seconds a black precipitate was observed as indication to CuO NPs. The precipitate was collected and dried.

$$CuSO_{4(aq)} + NaOH_{(l)} \xrightarrow{\text{Stabilizer}} CuO(NPs) \qquad \text{eq(3.1)}$$

The following parameters were studied: Particles size, the presence of surfactant, Temperature, NPs concentrations, pH, shaking and contact time with wastewater samples.

To control the size and the shapes of the nanoparticles, the synthesis was carried out at different constant temperatures (65, 75 and 85 °C), in the presence and absence of the stabilizer to obtain CuO NPs.

Zhu et al. prepared highly dispersed CuO nanoparticles by mixing copper acetate aqueous solution as a precursor with glacial acetic acid at 100 °C, black precipitate of CuO was observed by adding NaOH as reducing agent, the average size of CuO NPs produced was 6 nm [71]. Fathima *et al.* prepared stabilized CuO nanorods with sodium dodecylsulfate (SDS), cetyltrimethylammonium bromide (CTAB) and triton X-100 as anionic; cationic and neutral surfactants, respectively by fast and simple quick precipitation method. They found that, the ionic SDS surfactant has the strongest interaction of its negative charge with the cationic CuO NPs [79].

The advantage of our approach for CuO NPs synthesis is that can be considered as a green synthesis in which all materials used as reactant and all products are environmentally friendly.

The size and the shape of the obtained nanoparticles were characterized using XRD and SEM.

3.1.1 XRD characterization

XRD characterizations were carried out for the prepared CuO NPs, the obtained X-Ray diffractogram for all CuO with and without TOAB surfactant are shown in Fig 3.1 to Fig 3.6.

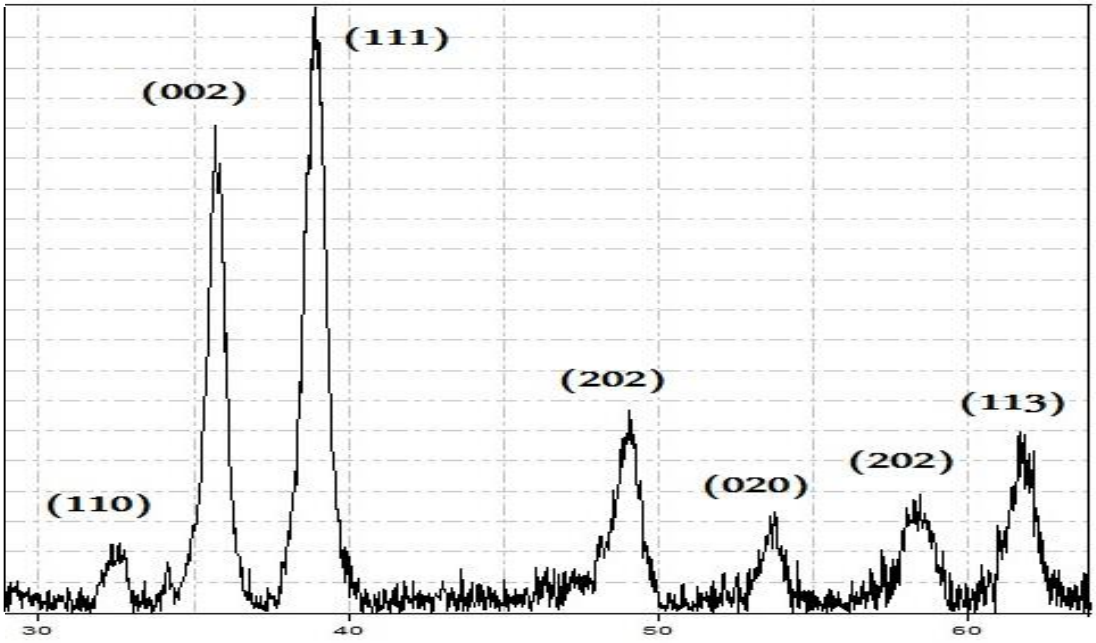


Fig 3.1: XRD pattern of CuO nanoparticles were prepared at 65 °C with TOAB surfactant.

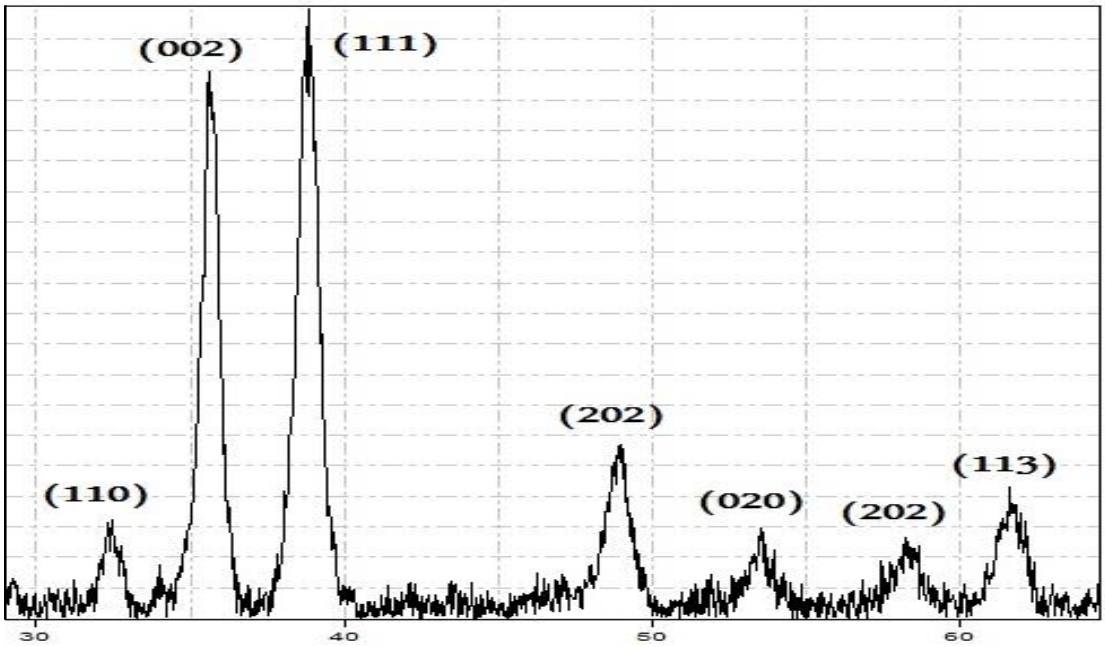


Fig 3.2: XRD pattern of CuO nanoparticles were prepared at 65 °C without TOAB surfactant

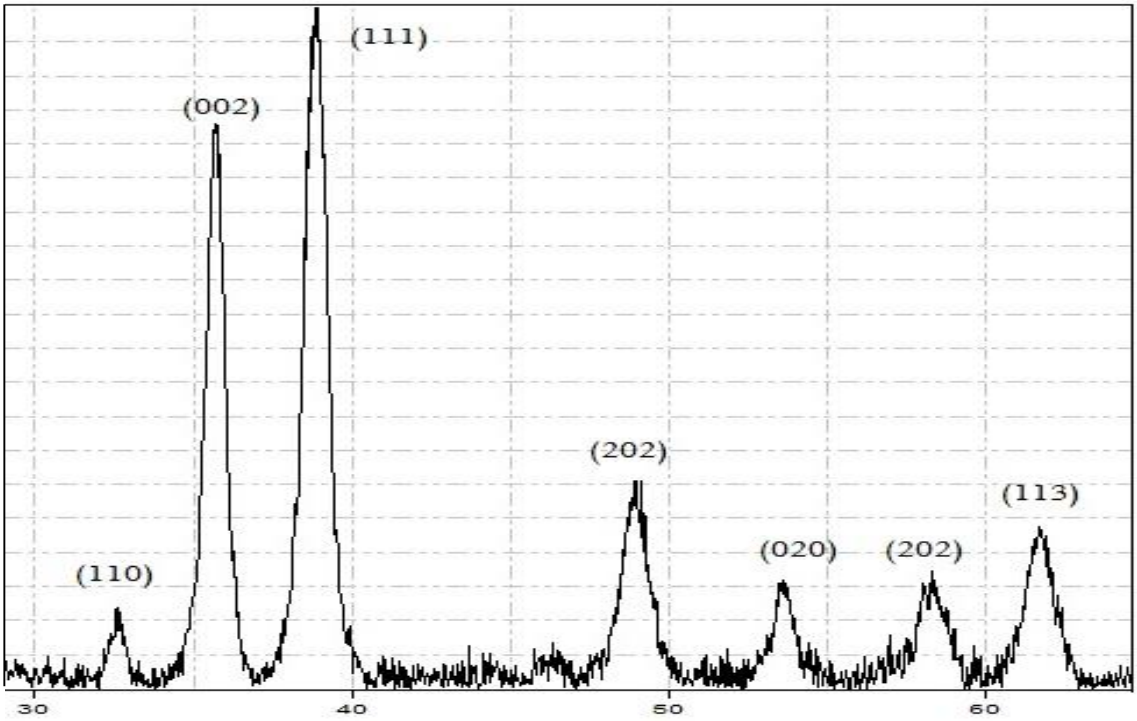


Fig 3.3: XRD pattern of CuO nanoparticles were prepared at 75 °C with TOAB surfactant

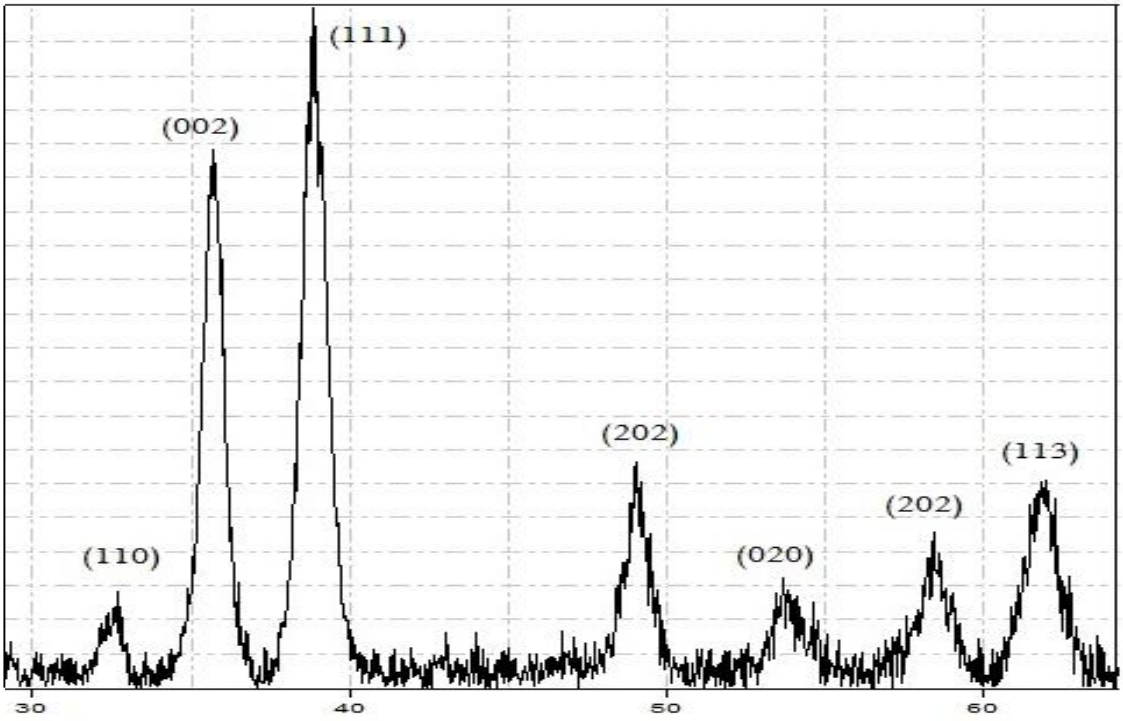


Fig 3.4: XRD pattern of CuO nanoparticles were prepared at 75 °C without TOAB surfactant

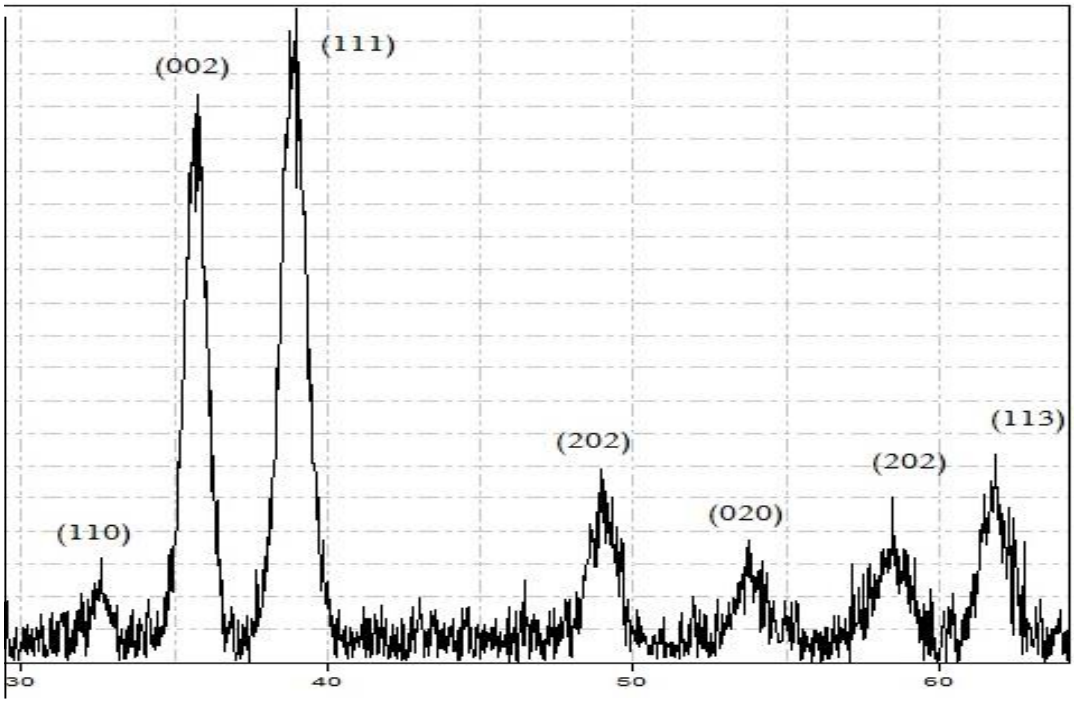


Fig 3.5: XRD pattern of CuO nanoparticles were prepared at 85 °C with TOAB surfactant

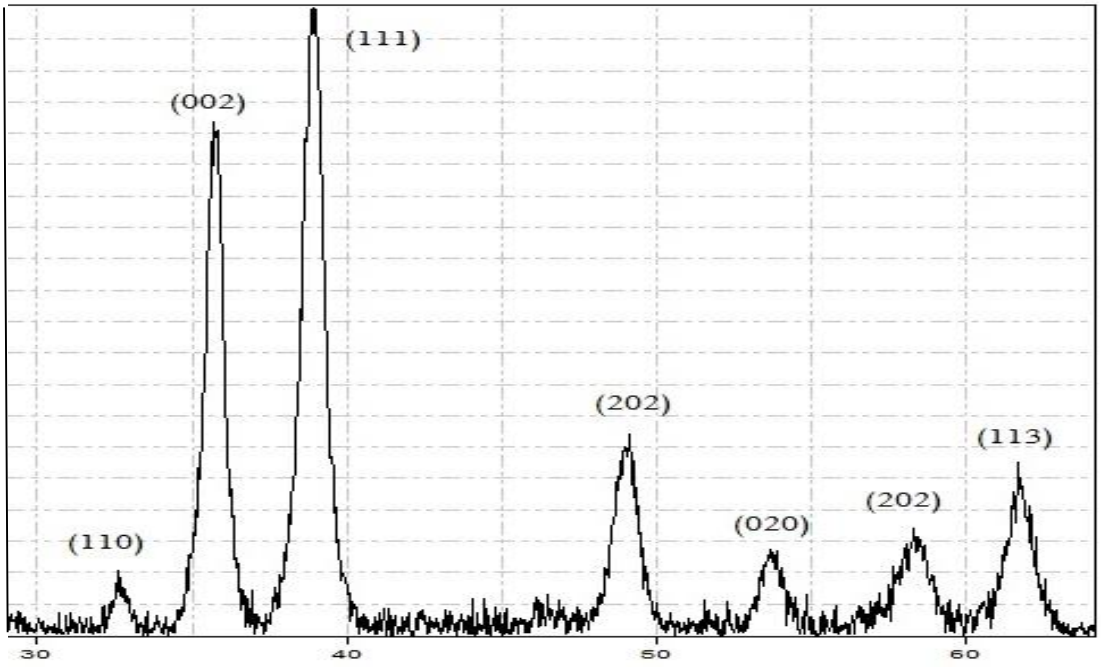


Fig 3.6: XRD pattern of CuO nanoparticles were prepared at 85 °C without TOAB surfactant

By applying Scherrer equation on the XRD pattern, the expected particle size can be determined:

$$D = K\lambda / (B\cos\theta)$$

Where D is the mean size of crystallites (nm), K is crystallite shape factor a good approximation is 0.9, λ is x-ray wavelength, B is full width at half the maximum (FWHM) in radians of the X-ray diffraction peak and θ is the Bragg angle [91].

Based on three different XRD peaks analysis, the mean size was found to be 11.5, 9.9 and 7.8 nm for CuO-TOAB stabilized NPs which were prepared at 65, 75 and 85 °C, respectively. While the sizes were found to be 12.4, 11.4 and 9.1 nm for CuO non-stabilized NPs were prepared at 65, 75, 85 °C, respectively which are obviously larger than the sizes obtained in the presence of the surfactant.

These results show that increasing the temperature during nanoparticles preparation lead to decrease in nanoparticle size. This can be explained as follows: the high temperature break the hydrogen bonds of the metastable copper hydroxide ($Cu(OH)_2$) to transform into CuO. This complex was formed when $CuSO_4.5H_2O$ dissolved in water to produce hexaaqua copper(II) ion $[Cu(H_2O)_6]^{+2}$ followed by formation of $Cu(OH)_2$ after adding NaOH to the solution [77, 97].

The sizes results of CuO-TOAB stabilized NPs and CuO non-stabilized NPs with different temperature are summarized in Table 3.1. The size of CuO-

TOAB stabilized NPs are smaller than CuO non-stabilized NPs that were prepared at the same temperature. Therefore, the surfactant may have a role in controlling the CuO NPs size according to its role in preventing the undesired aggregation of atoms during nanoparticles preparation [92].

Table 3.1: The sizes results of CuO-TOAB stabilized NPs and CuO non-stabilized NPs at different temperatures

Temperature (ºC)	CuO-TOAB stabilized sizes (nm)	CuO non-stabilized NPs sizes (nm)
65	11.5	12.4
75	9.9	11.4
85	7.8	9.1

Azam et al. prepared different size of CuO NPs by dissolving 1:1 molar ratio of $Cu(NO_3)_2 \cdot 3H_2O$ and citric acid at different temperatures. The obtained sizes were 20, 21, 25 and 27 nm at 400°C, 500°C, 600°C, and 700°C, respectively [90]. These results are in contrast with our results.

On the other hand *Wu et al.* prepared well dispersed CuO nanoparticles by dissolving copper(II) nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$) in N,N-Dimethylacet amide (DMAC) at 100 °C using NaOH as reducing agent, they studied the effect of the temperature of the added NaOH (20 to 140 °C). They found that, the NPs size decreases with increasing the temperature as long as the temperature was below 100 °C and the size of NPs increases with increasing temperature when the temperature is above 100 °C. The size of NPs was 11.0 nm, 9.0 nm, 6.7 nm and 8.7 nm when the preparation temperature was 20, 55, 100, and 140 °C, respectively [77].

By comparing our work with Azam *et al* and *Wu et al.* work, our results are better than their results in many aspects, our CuO NPs source was $CuSO_4.5H_2O$ which is more than three times cheaper than $Cu(NO_3)_2 \cdot 3H_2O$, also the preparation temperatures we used were less than Azam *et al.* and give smaller size NPs. Wu *et al.* results are in accordance with our results in that the obtained CuO NPs size decreases with increasing temperature until 100 °C.

As reported in previous studies, X-ray structural analysis of the prepared samples (Fig 3.1 to Fig 3.6) confirmed copper oxide CuO nanoparticles monoclinic structures [93].

3.1.2 SEM characterization

The shape and size of NPs were investigated by SEM techniques, Fig 3.7 to Fig 3.9 show the SEM images of all CuO nanoparticles samples which were prepared with and without surfactant at different temperature.

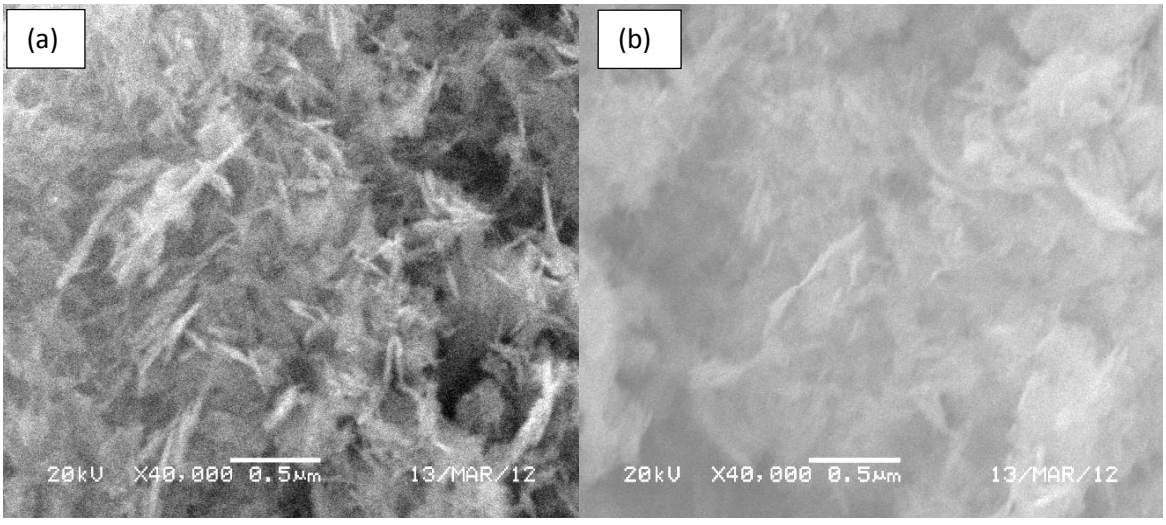


Fig 3.7: The SEM images of CuO nanoparticles prepared at 65 °C a) with TOAB surfactant b) without TOAB surfactant.

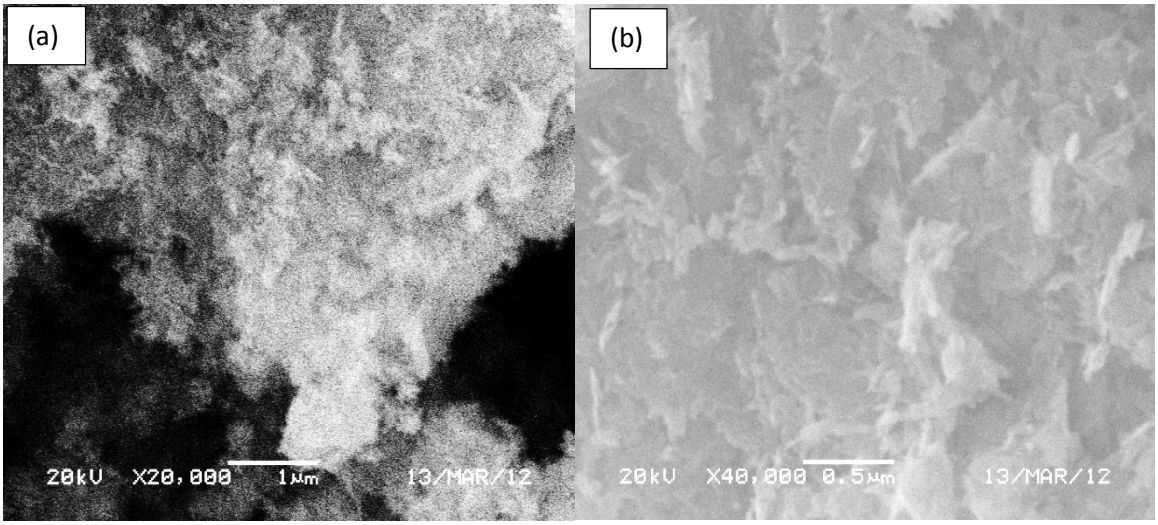


Fig 3.8: The SEM images of CuO nanoparticles prepared at 75 °C a) with TOAB surfactant b) without TOAB surfactant.

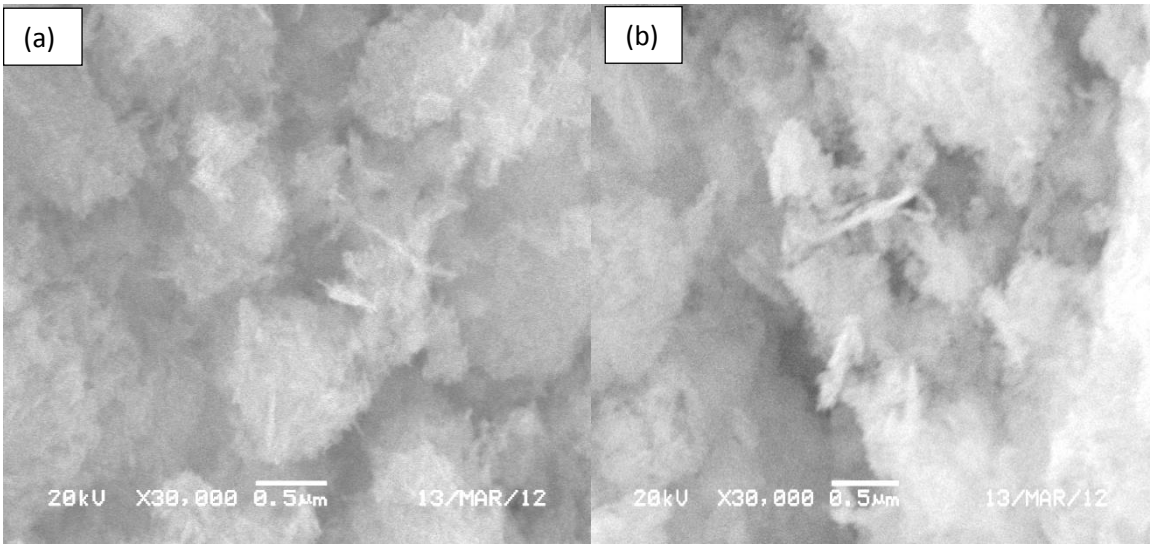


Fig 3.9: The SEM images of CuO nanoparticles prepared at 85 °C a) with TOAB surfactant b) without TOAB surfactant

Rody-stick shape was recorded for all CuO NPs with and without surfactant samples, but it looks more regular for CuO-TOAB stabilized NPs.

Wu et al. reported that the change of NaOH adding temperatures leads to change in CuO NPs shape, its seems spindly shape at small temperature and goes to spherical shape at 100 °C [77].

However, in this work, rody-stick shape obtained for all CuO NPs that prepared at different temperatures; 65, 75 and 85 ºC.

3.2 Antibacterial activity study

Antibacterial activity of CuO NPs with and without TOAB surfactant was studied on TC, FC and *Enterococcus faecalis* (*E. faecalis*) in wastewater. Many parameters were studied to obtain the optimum wastewater disinfection conditions, these parameters were size of nanoparticles with and without surfactant, nanoparticles concentrations, contact time, pH, shaking and temperature of wastewater effect.

The highest bacterial growth was at 25 ºC followed by 35 ºC and the least was at 15 ºC for all studied bacterial indicators, TC, FC and *E. faecalis*. This result is in consistent with an earlier study on wastewater that showed the maximum bacterial growth at 20 ºC [88]. In another more recent study that indicated optimal growth was at 30 ºC at extended incubation temperature, the shorter incubation of about 6 days showed the maximum growth was at 20 ºC [89]. Therefore, all the incubation temperature used throughout this study was at 25 ºC

3.2.1 Antibacterial activity of different concentrations CuO NPs with and without TOAB

Antibacterial activities of different NPs concentrations were conducted on wastewater to monitor the bacterial degradation percent for each concentration. In this study CuO(4) was chosen because of its superior antibacterial activity more than other CuO non-stabilized NPs samples according to preliminary tests

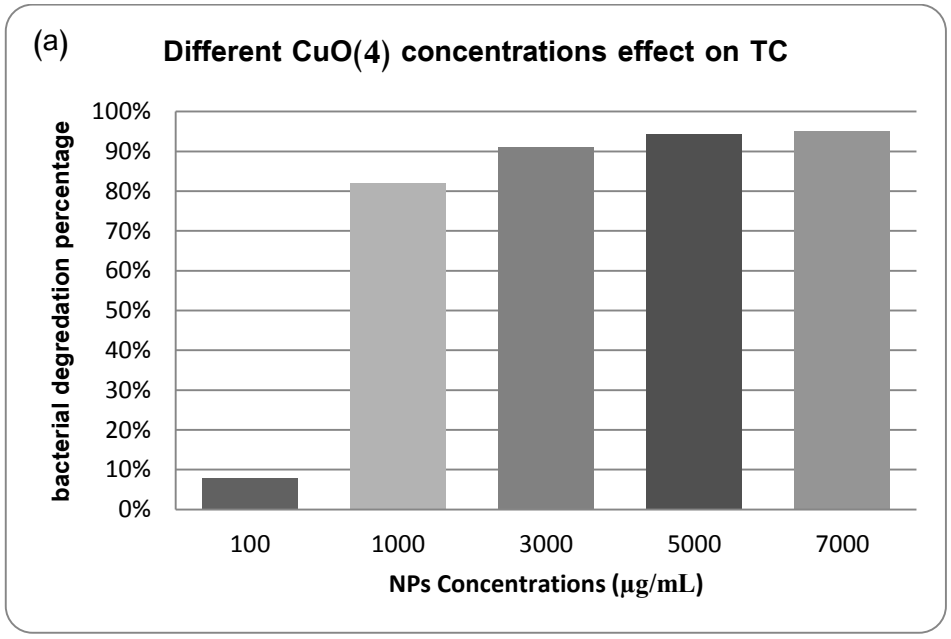
and has been confirmed in the next section (3.3.2), Different CuO(4) NPs concentrations were chosen $1x10^2$, $1x10^3$, $3x10^3$, $5x10^3$ and $7x10^3$µg/mL. For CuO NPs with surfactant the chosen concentrations was 10, 100, 300 and 500 µg/mL of CuO-TOAB(3), that were chosen due to its superior antibacterial activity of other CuO-TOAB stabilized NPs according to preliminary tests and has been confirmed in the next section (3.2.2).

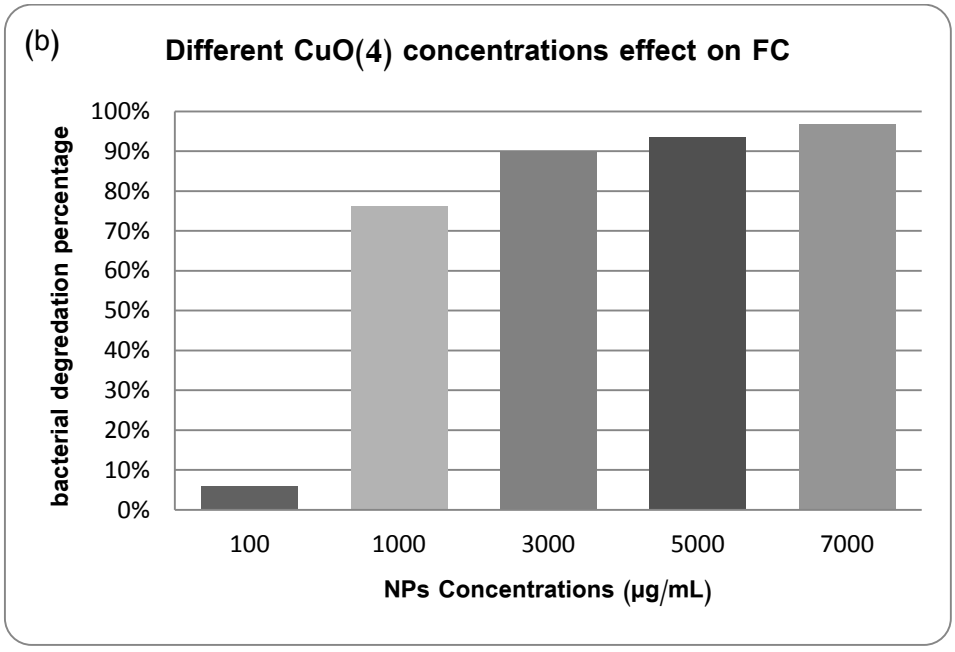
The results of TC, FC and *E. faecalis* bacterial degradation by the different concentrations CuO(4) treated wastewater are listed in Table 3.2 and Fig 3.10 (a) to (c),

Table 3.2: CuO(4) NPs different concentrations bacterial growth degradation percentage.

Concentrations	10^2	10^3	$3x10^3$	$5x10^3$	$7x10^3$
TC	8%	82%	91%	94%	95%
FC	6%	76 %	90%	93%	97%
E. faecalis	7%	85 %	94 %	97%	98%

Bacterial degradation was consistent for the used bacterial degradation indicators; TC, FC and *E. faecalis* and all were less than 1000 µg/mL. The noticed percentage degradation was very close for all used indicators. However, there is a slightly higher degradation percentage for *E. faecalis* in comparison to TC and FC, that could be correlated to the bacterial cell wall structure of gram-positive *E. faecalis* bacteria and the gram-negative bacteria represented by TC and FC (Table 3.2), also Fig.3.11 shows 0 µg/mL and $7x10^3$ µg/mL concentrations effect of CuO(4) on all tested bacteria.


(a)
Different CuO(4) concentrations effect on TC
bacterial degredation percentage
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100
1000
3000
5000
7000
NPs Concentrations (µg/mL)


(b)
Different CuO(4) concentrations effect on FC
bacterial degredation percentage
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100
1000
3000
5000
7000
NPs Concentrations (µg/mL)

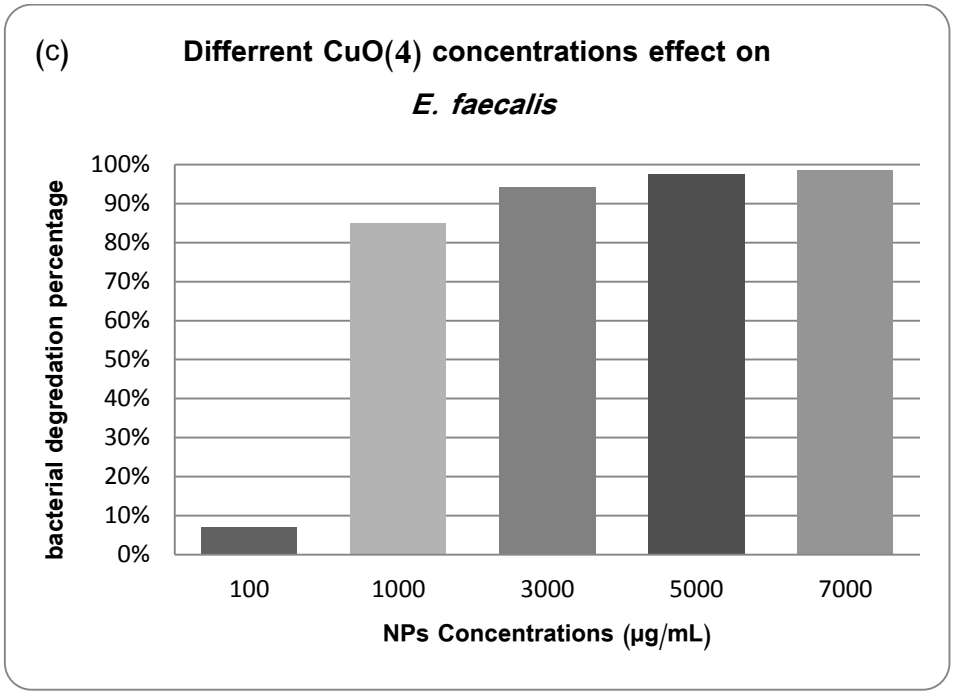


Fig 3.10: CuO(4) NPs different concentrations bacterial growth degradation percentage using a) TC, b) FC and c) *E. faecalis* bacteria.

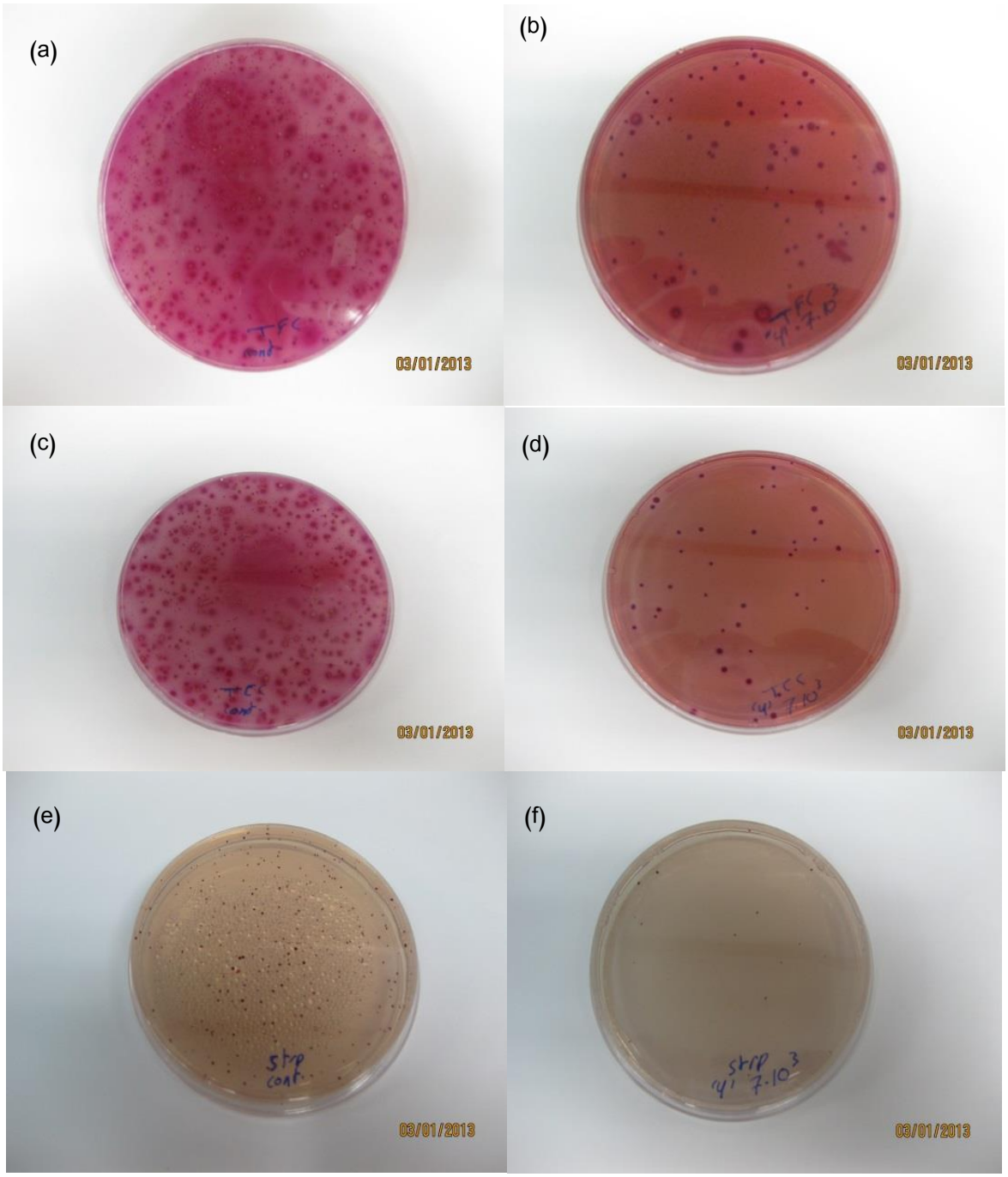
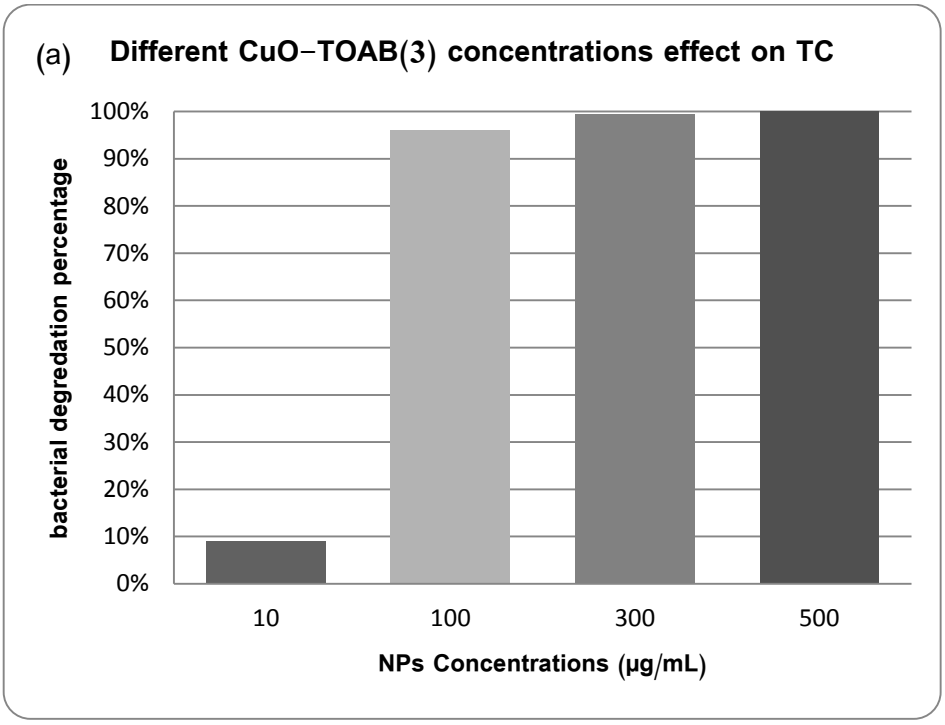
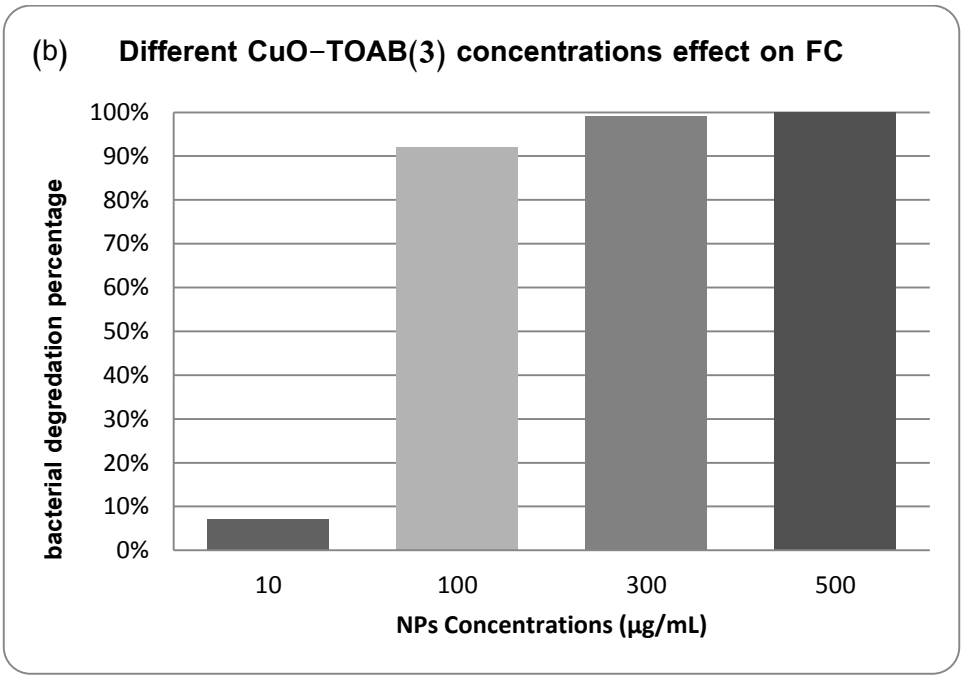


Fig.3.11: Shows 0 µg/mL effect of CuO(4) NPs on a) FC c) TC and e) *E. faecalis.* And $7x10^3$ µg/mL of CuO(4) NPs effect on b) FC d) TC and f) *E. faecalis.*

The results of bacterial growth inhibition percentage for TC, FC and *E. faecalis* treated with different concentrations of CuO-TOAB(3) are shown in Fig 3.12 and Table 3.3.





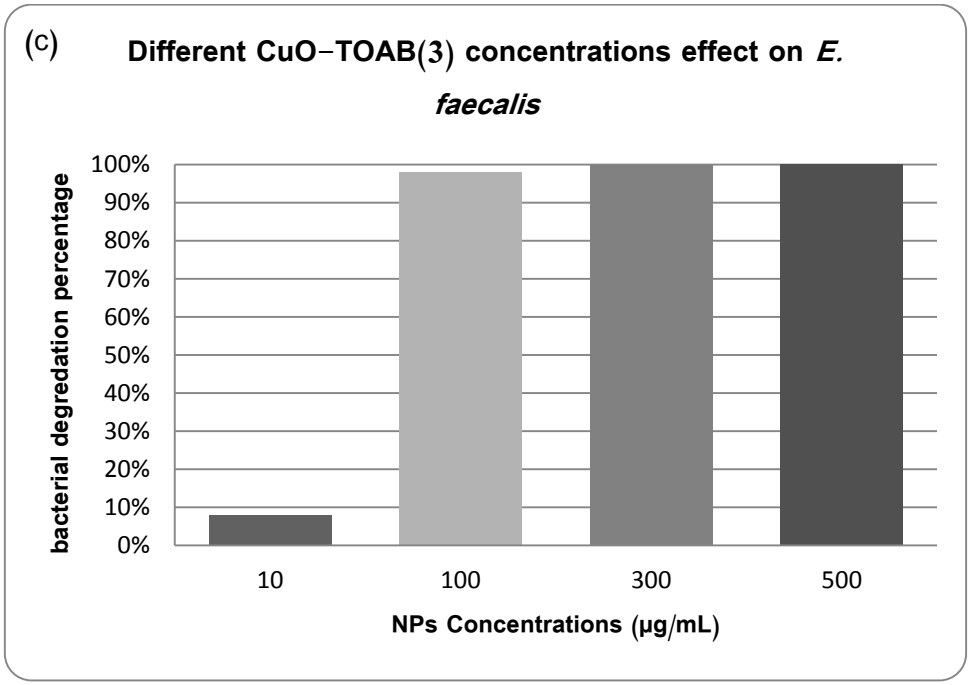


Fig 3.12: The different CuO-TOAB(3) concentrations effect on a) TC, b) FC and c) *E. faecalis*.

Table 3.3 shows antibacterial inhibition growth percentage that treated with different concentrations of CuO-TOAB(3) NPs. Also Fig.3.11 shows 0 µg/mL and $5x10^2$ µg/mL concentrations effect of CuO-TOAB(3) on all tested bacteria.

Table 3.3: TC, FC and *E. faecalis* inhibition growthpercentage that treated with CuO-TOAB(3) NPs.

CuO-TOAB(3)conc.	10	10^2	$3x10^2$	$5x10^2$
TC	8%	96 %	99 %	100 %
FC	7%	92 %	99 %	100 %
E. faecalis	9%	98 %	99 %	100 %

As indicated in the bacterial degradation for CuO NPs, bacterial degradation of CuO- TOAB stabilized NPs were consistent for the used bacterial

degradation indicators that were less than 100 µg/mL. Moreover, bacterial degradation percentage of CuO-TOAB stabilized NPs were again higher in gram positive bacteria against *E. faecalis* in comparison with gram negative TC and FC indicators (Table 3.3).

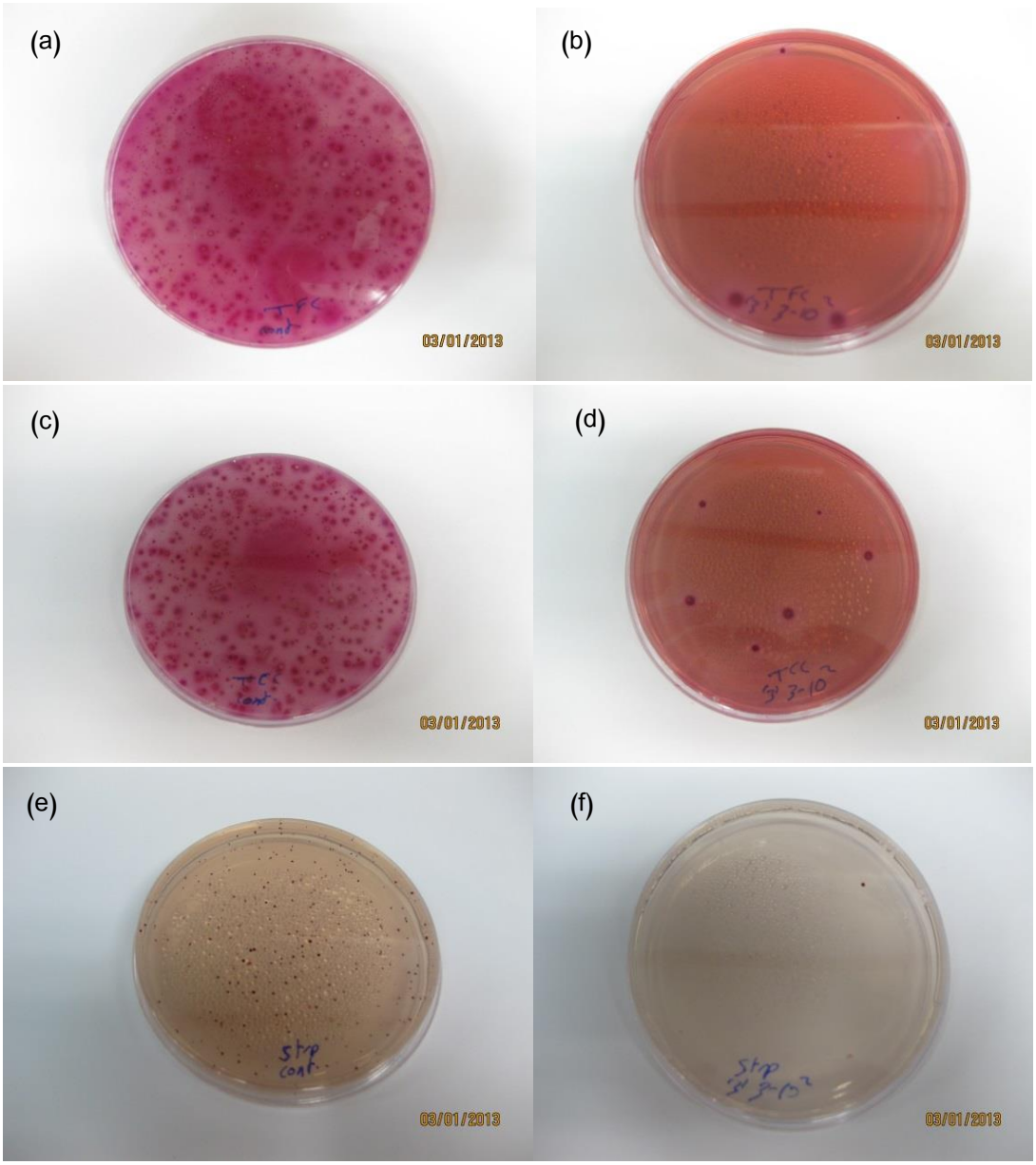


Fig.3.13: Shows 0 µg/mL effect of CuO-TOAB(3) NPs on a) FC c) TC and e) *E. faecalis.* And $3x10^2$ µg/mL of CuO-TOAB(3) NPs effect on b) FC d) TC and f) *E. faecalis.*

As shown in Table 3.2 and 3.3, antibacterial activity used indicators in this study, TC, FC and *E. faecalis*, showed a very close NPs concentration response for both CuO with and without TOAB surfactant. The bacterial degradation rate begin at about 100 and 10 µg/mL for CuO(4) and CuO-TOAB(3), respectively. As well as, antibacterial activity were increased with increasing NPs concentrations.

In previous studies, CuO NPs MBC against *E. coli* was 250 µg/mL [94] and 30 µg/mL [90]. In the later study, Azam *et al*. showed a wide range of MBCs from 30 – 95 reflecting the CuO NPs size in the range from 20 – 27 nm. Baek *et al.* reported the lowest observed effect concentration of CuO NPs against *E. coli* that were 30 µg/mL [81]. Our study result, using FC as indication for *E. coli*, was comparable even much better than these reported results as the noticed MBC, about 100 and 10 µg/mL for CuO(4) and CuO-TOAB(3). To our best of knowledge, there is no data concerning the usage of CuO NPs on sewage samples.

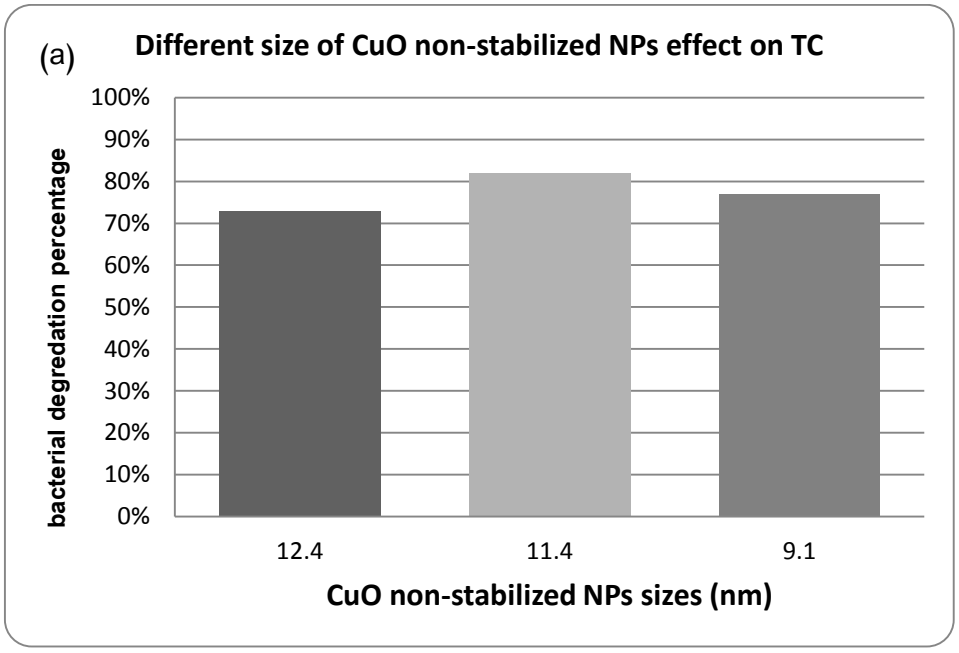
3.2.2 CuO NPs size and TOAB surfactant antibacterial effect

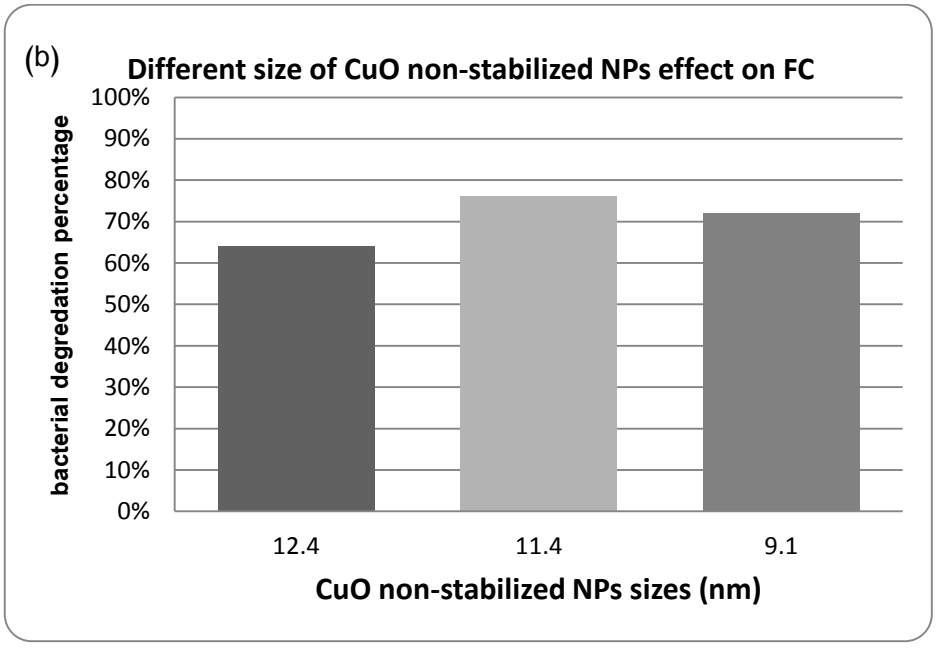
Different particles size with and without TOAB surfactant were used to study their TC, FC and *E. faecalis* bacteria degradation percentage after wastewater disinfection.

Fig.3.14 (a) to (c) shows the bacterial degradation percentage of CuO non-stabilized NPs (10^3 µg/mL); CuO(2), CuO(4) and CuO(6) that represent 12.4, 11.4 and 9.1 nm NPs size, respectively.

Table 3.4: Different CuO NPs size without TOAB surfactant inhibition growth percentage rate on TC, FC and *E.faecalis*.

NPs different size without TOAB surfactant (nm)	12.4	11.4	9.1
TC	73%	82%	77%
FC	64%	76%	72%
E. faecalis	75%	85%	78%





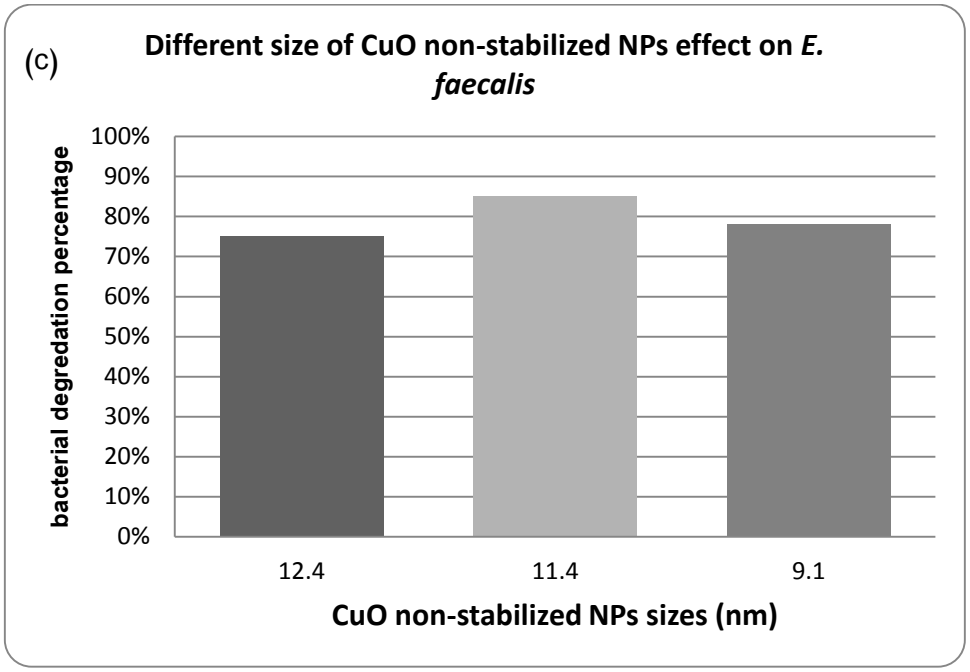


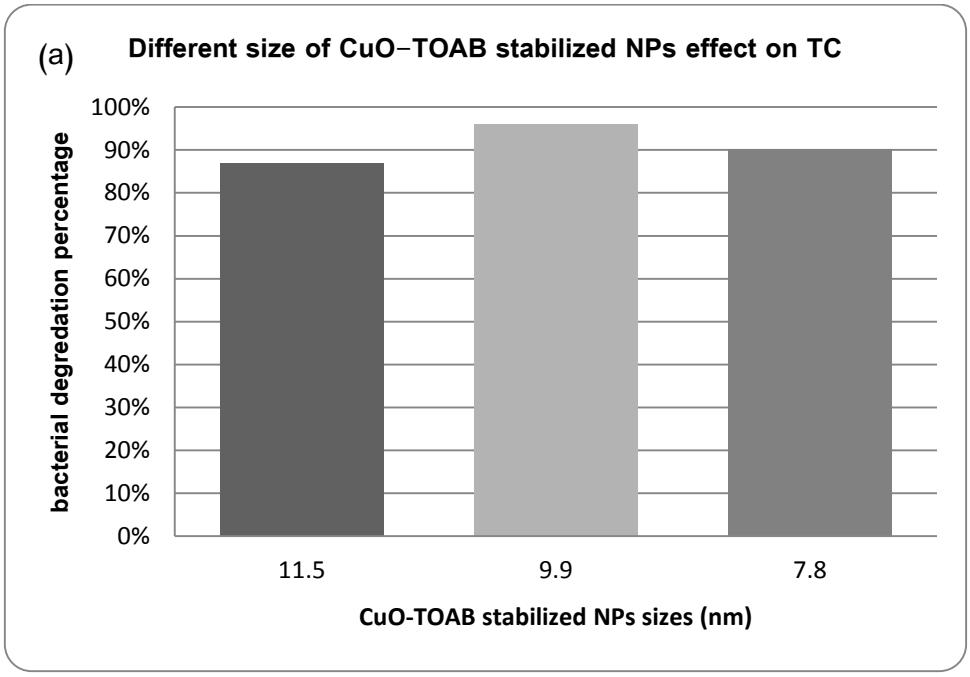
Fig. 3.14: CuO non-stabilized NPs size effect on a) TC b) FC and c) *E. faecalis* bacteria

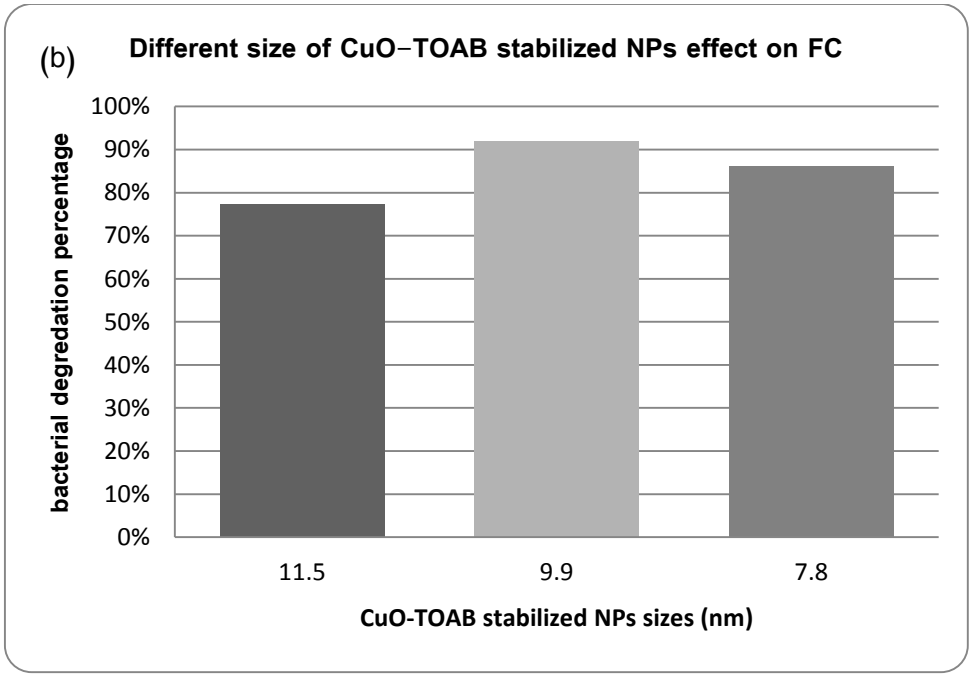
Table 3.4 shows the TC, FC and *E. faecalis* inhibition growth percentage after treated with different NPs size without TOAB surfactant.

Fig.3.15 (a) to (c) and Table 3.5 show the bacterial degradation percentage of CuO-TOAB stabilized NPs (10^2 µg/mL); CuO-TOAB(1), CuO-TOAB(3) and CuO-TOAB(5) that represent 11.5, 9.9 and 7.8 nm NPs size, respectively.

Table 3.5: Different NPs size with TOAB surfactant inhibition growth percentage rate on TC, FC and *E. faecalis.*

NPs different size withTOAB surfactant (nm)	11.5	9.9	7.8
TC	86%	96%	90%
FC	77%	92%	86%
E. faecalis	89%	98%	92%


(a)
Different size of CuO−TOAB stabilized NPs effect on TC
bacterial degredation percentage
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
11.5
9.9
7.8
CuO-TOAB stabilized NPs sizes (nm)


(b)
Different size of CuO−TOAB stabilized NPs effect on FC
bacterial degredation percentage
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
11.5
9.9
7.8
CuO-TOAB stabilized NPs sizes (nm)

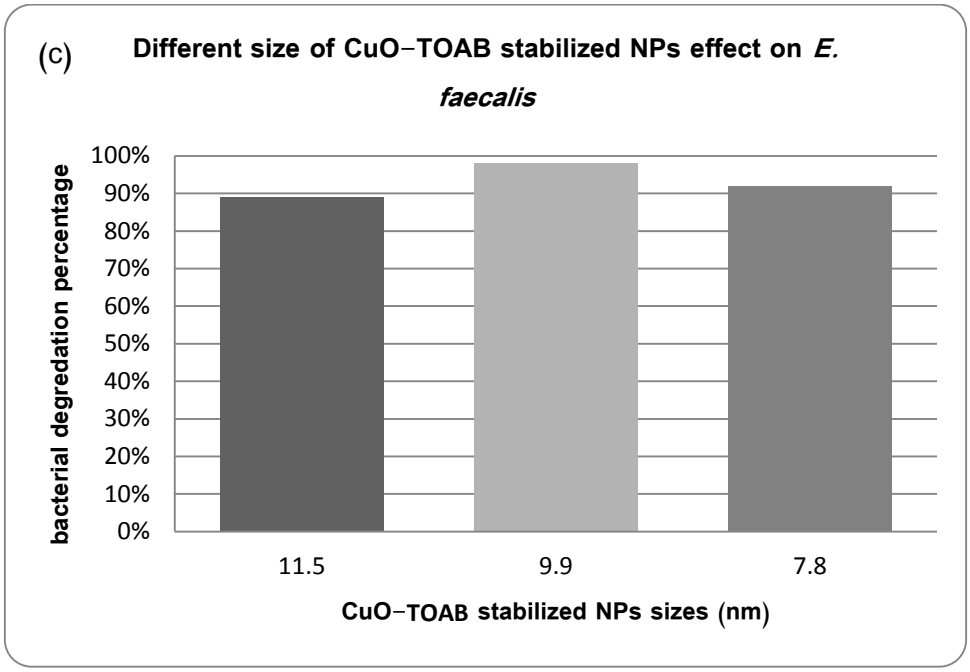


Fig. 3.15: CuO NPs size with surfactant effect on a) TC b) FC and c) *E. faecalis*.

Previous studies indicated that decreasing CuO NPs size usually increases the antimicrobial activity against both Gram-positive and Gram-negative bacterial strains according to NPs surface to volume ratio which increases with decreasing the size [90].

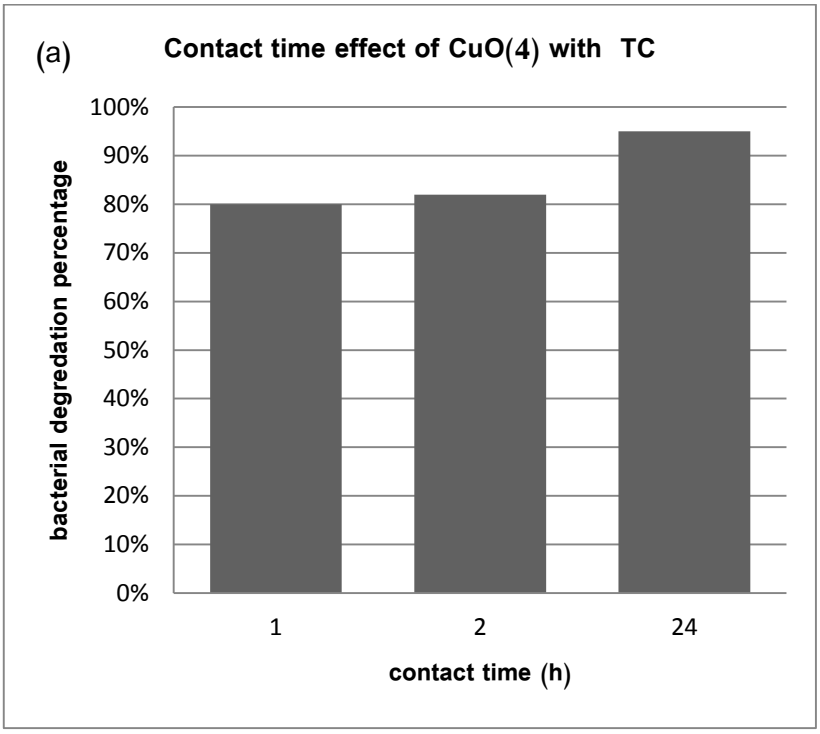
Interestingly, this study results (Table 3.4 and 3.5) showed that the highest antibacterial activity were for the medium size for both CuO with TOAB (9.9 nm) and without TOAB surfactant (11.4 nm). To the best of our knowledge the only available article that studied the size effect of CuO reached only 20 nm CuO NPs size with the highest antibacterial activity among the studied NPs size that goes with the notion of decreasing NPs size will increase the antibacterial activity [90]. However, our work reached a smaller size of NPs,

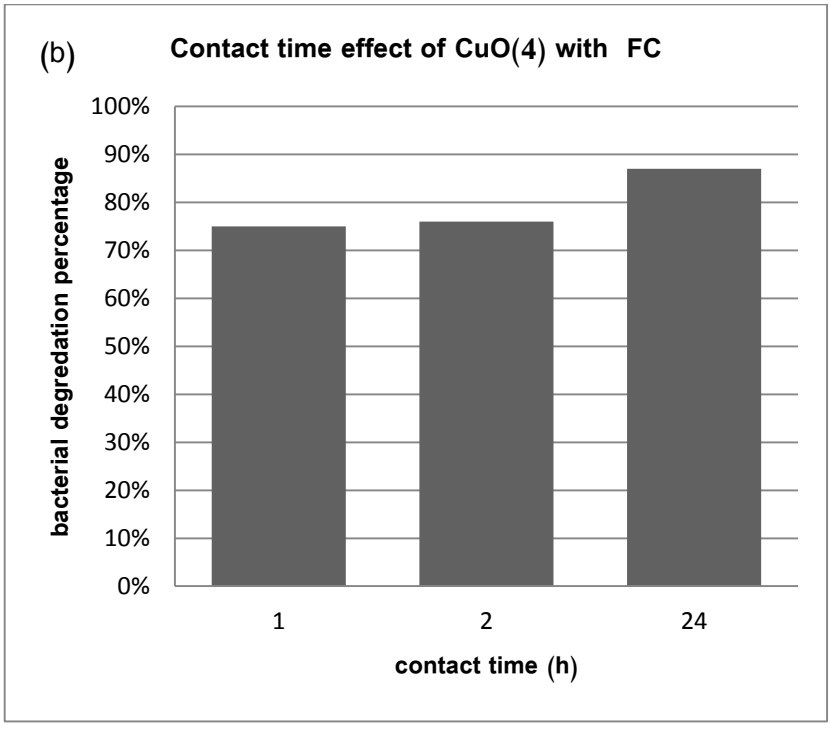
less than 12.4 and 11.5 of CuO without TOAB and CuO with TOAB, respectively.

3.2.3 Antibacterial activity of CuO NPs with different contact time

The antibacterial activity of CuO NPs was studied to determine the most appropriate contact time. The effect was studied for two samples type: CuO(4) without surfactant (10^3 µg/mL) and CuO-TOAB(3) with stabilizer(10^2 µg/mL). Antibacterial activity was studied at different contact time of 1, 2 and 24h. The results for CuO(4) NPs without surfactant are shown in Fig 3.15 and Table 3.6. Fig 3.16 and Table 3.7, shows the results obtained for CuO-TOAB(3) NPs with TOAB surfactant.

Antibacterial activity of CuO NPs without TOAB surfactant after 1, 2, 24 hours contact time of TC were 80, 82 and 95%, respectively (Fig 3.16a); FC were 75, 76 and 87%, respectively (Fig 3.16b); and *E. faecalis* were 80, 85 and 100% respectively (Fig 3.16c, Table 3.6).


(a)
Contact time effect of CuO(4) with TC
bacterial degredation percentage
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
2
24
contact time (h)


(b)
Contact time effect of CuO(4) with FC
bacterial degredation percentage
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
2
24
contact time (h)

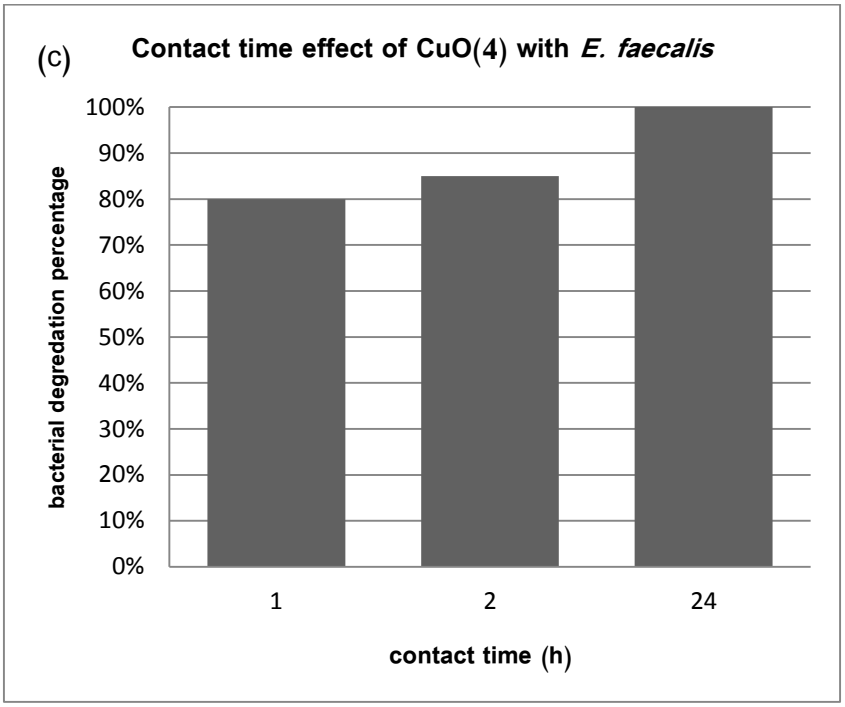
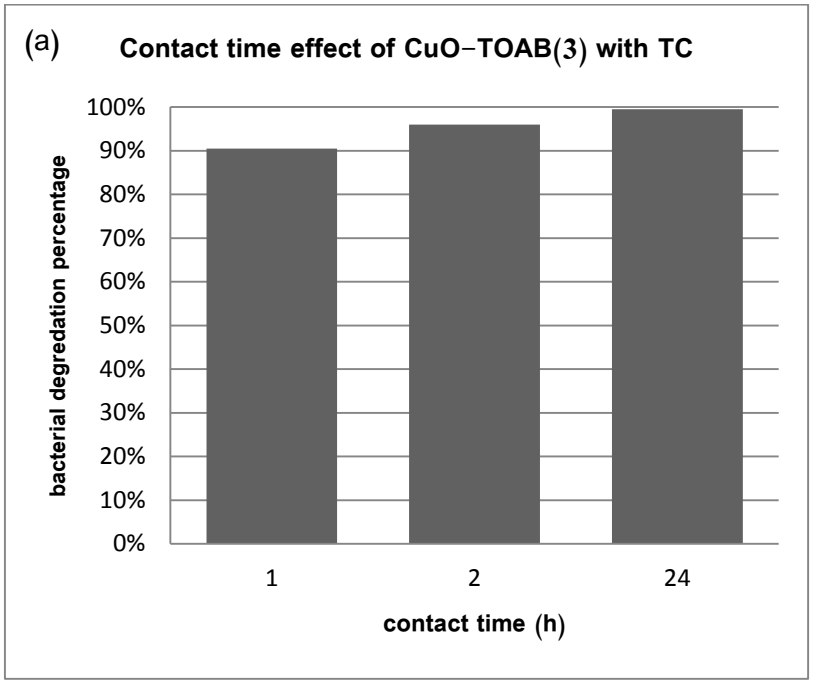


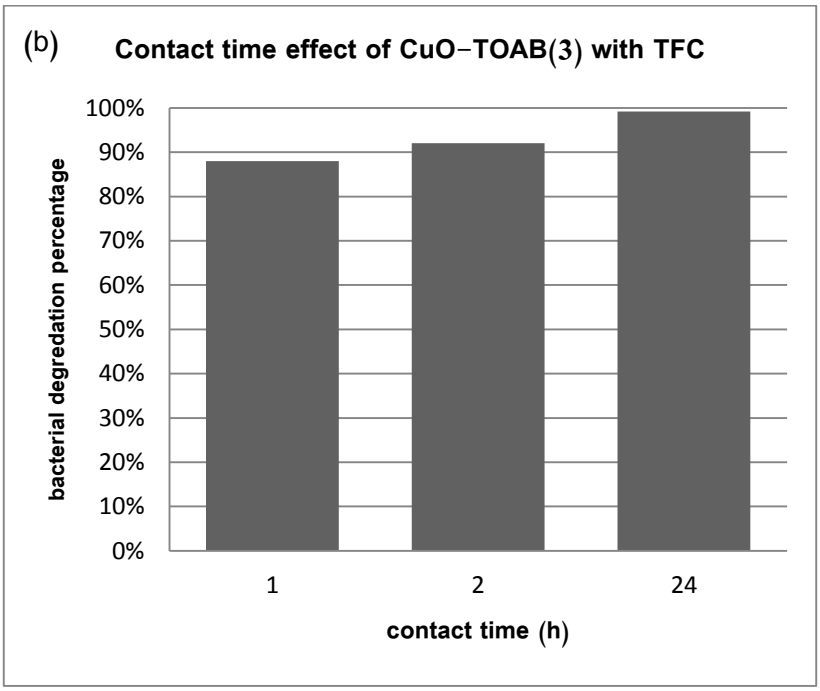
Fig 3.16: 1, 2 and 24 h contact time effect of CuO(4) on a) TC b) FC c) *E. faecalis*

Table3.6: Inhibition growth rate of TC, FC and *E. faecalis* bacteria using CuO(4) NPs

Time (h)	1	2	24
TC	80%	82%	95%
FC	75%	76%	87%
E. faecalis	80%	85%	100%

Antibacterial activity of CuO NPs stabilized with TOAB surfactant after 1, 2, 24 hours contact time of TC were 90, 96 and 99%, respectively (Fig 3.17a); FC were 88, 92 and 99%, respectively (Fig 3.17b); and *E. faecalis* were 91, 98 and 100% respectively (Fig 3.17c, Table 3.7).


(a)
Contact time effect of CuO−TOAB(3) with TC
bacterial degredation percentage
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
2
24
contact time (h)


(b)
Contact time effect of CuO−TOAB(3) with TFC
bacterial degredation percentage
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
2
24
contact time (h)

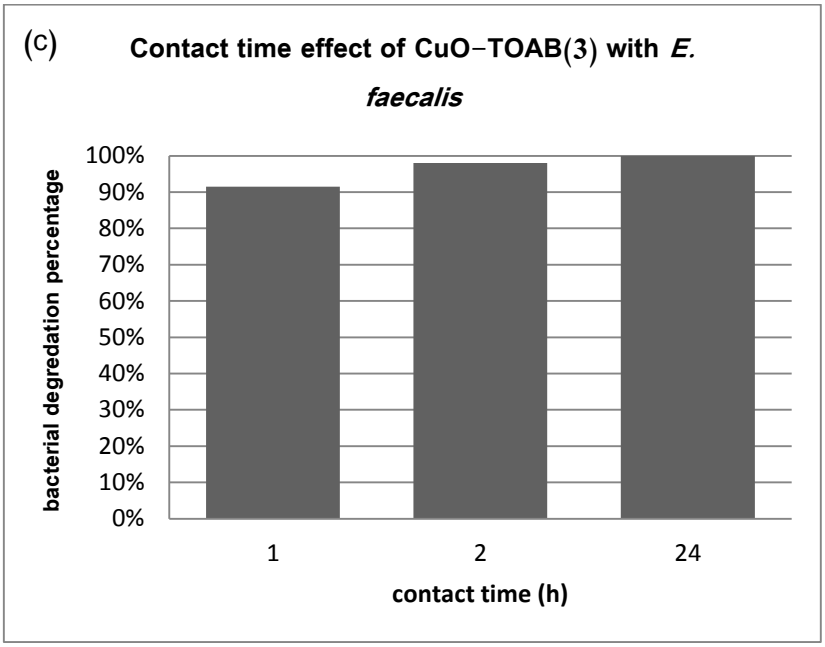


Fig 3.17: The effect of contact time; 1, 2 and 24 h of CuO-TOAB(3) with a) TC b) FC c) *E. faecalis*

Table3.7: Inhibition growth rate of TC, FC and *E. faecalis* bacteria using CuO-TOAB(3) NPs

Time (h)	1	2	24
TC	90.5%	96%	99.5%
FC	88%	92%	99.2%
E. faecalis	91.5%	98%	100%

As indicated earlier in this study, CuO-TOAB stabilized NPs showed a higher antibacterial activity at all studied contact time 1, 2, 24h, in comparison to the CuO non-stabilized NPs (Table 3.6, Table 3.7). The results showed that, increases contact time has increased the antibacterial activity of both CuO NPs with and without TOAB surfactant. Generally, the difference in contact time antibacterial effect was small in both CuO NPs with and without TOAB.

However, the antibacterial activity difference was slightly higher in CuO NPs without TOAB, which give the TOAB a better advantage to be used for wastewater treatment at shorter contact time.

3.2.4 Temperature effect

Temperature is one of the most important factors affecting bacterial growth in wastewater. Palestine annual weather temperature almost range from 10-35 °C, that may reflect the wastewater temperature, however the wastewater temperature will probably have much narrower temperature range, as a reflection of the slow water response to the weather temperature and the wastewater samples are of human sources with constant body temperature. Therefore, the effect of different temperatures; 15, 25 and 35 °C, were studied as a factor in the antibacterial activity of CuO NPs with and without TOAB surfactant (Table 3.8, 3.9 and 3.10).

Table 3.8: Antibacterial activity of CuO-TOAB(3) and CuO(4) on TC with different temperatures.

NPs/ temp. (°C)	15	25	35
CuO-TOAB(3) (10^2µg/mL)	26%	96%	19%
CuO(4) (10^3µg/mL)	79%	82%	100%

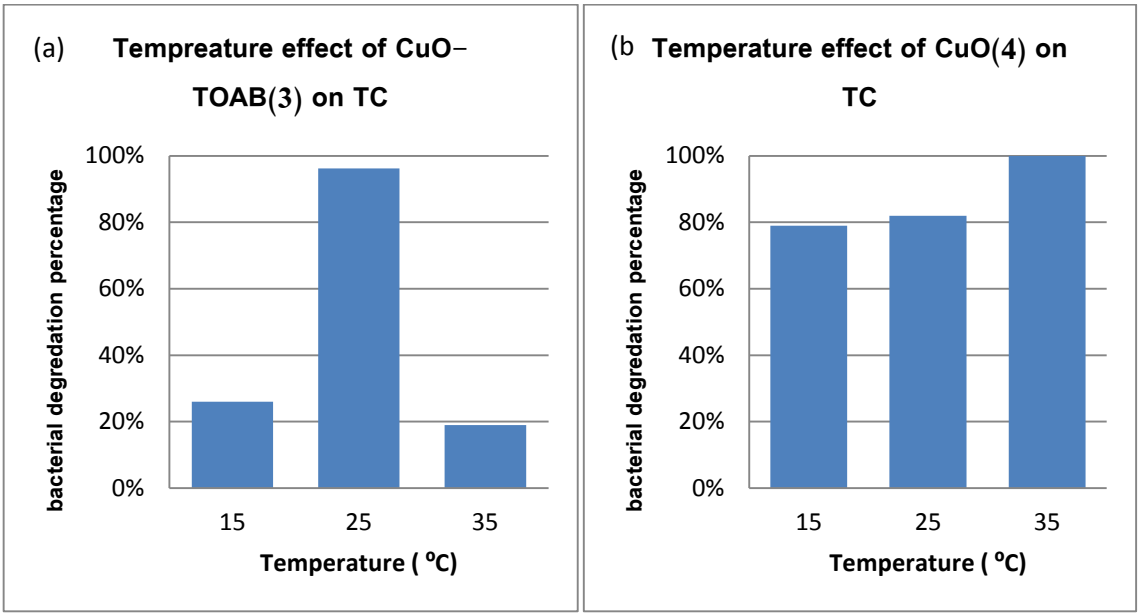


Fig 3.18: The effect of wastewater temperature (15, 25 and 35 ºC) on degradation percentage of TC using a) CuO-TOAB(3) and b) CuO(4)

Even the bacterial count of untreated original wastewater samples, which were used as control, at 35 °C to 25 °C incubation temperature was of about 50%, i.e, the count at 25 °C was double of the 35 °C. As shown in Table 3.8, 3.9 and 3.10, the maximum bacterial degradation percentage of about 100% was seen in CuO NPs without TOAB surfactant stabilization at 35 °C. Interestingly, this is in contrary to our preliminary investigation that the indicated maximum bacterial growth was 25 ºC, that were used as the optimal treatment temperature throughout the previous sections. The other temperature of 15 ºC and 25 ºC of CuO NPs without TOAB showed a closer result, however it was higher at 25 ºC, that could be explained by the metabolically active bacterial isolates at 25 ºC.

Table 3.9: Antibacterial activity of CuO-TOAB(3) and CuO(4) on FC with different temperatures.

NPs/ temp. (°C)	15	25	35
CuO-TOAB(3) (10^2µg/mL)	28%	91%	18%
CuO (4) (10^3µg/mL)	76%	78%	100%

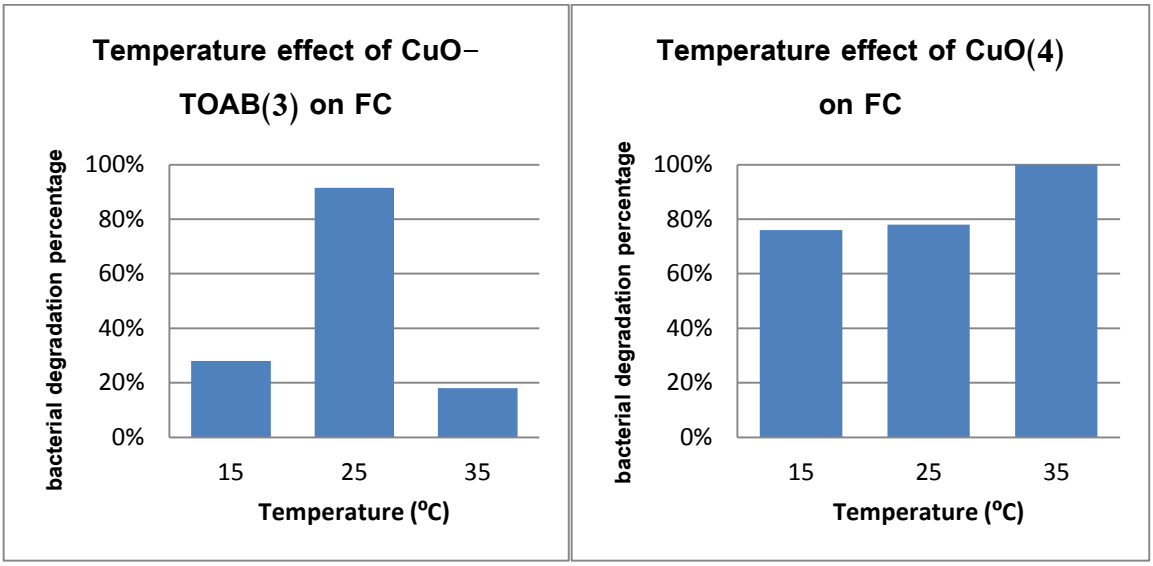


Fig 3.19: The effect of wastewater temperature (15, 25 and 35 ºC) on degradation percentage of FC using a) CuO-TOAB(3) and b) CuO(4)

In comparison to CuO NPs without TOAB surfactant, CuO NPs stabilized with TOAB (Table 3.8 -3.10 and Figure 3.18-3.20) showed the maximum bacterial degradation percentage at 25 ºC with 96, 91 and 98% for TC, FC and *E. faecalis*, respectively. Unexpectedly, there was a tremendous reduction in the bacterial degradation percentage at 15 ºC and 35 ºC. Moreover, the highest degradation was at 35 ºC. This result could be explained by that the surfactant highest effect with maximum metabolically active bacterial count at 25 ºC.

Table 3.10: Antibacterial activity of CuO-TOAB(3) and CuO(4) on *E. faecalis* with different temperatures.

NPs/ temp. (°C)	15	25	35
CuO-TOAB(3)(10^2µg/mL)	54%	98%	94%
CuO (4) (10^3µg/mL)	80%	87%	99%

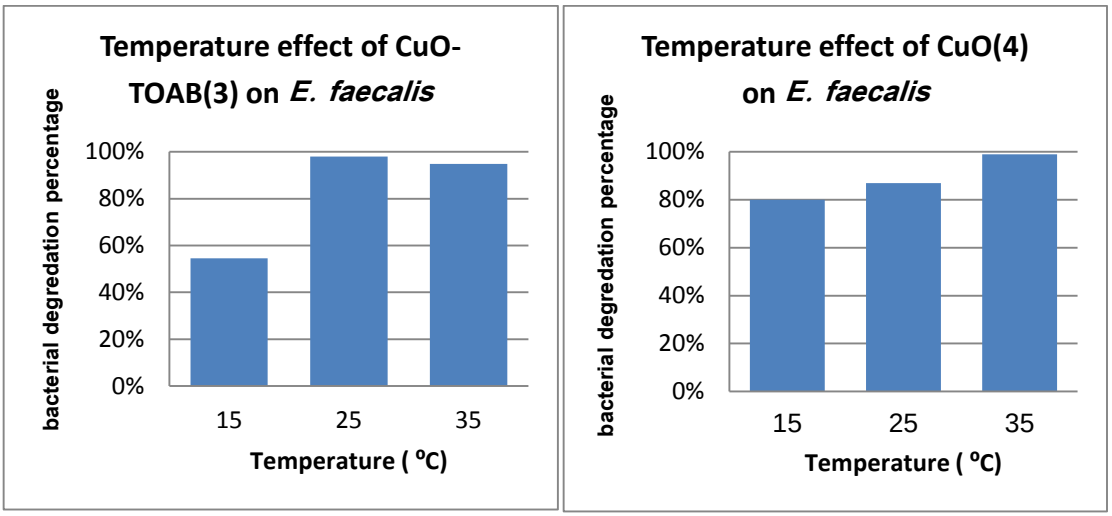
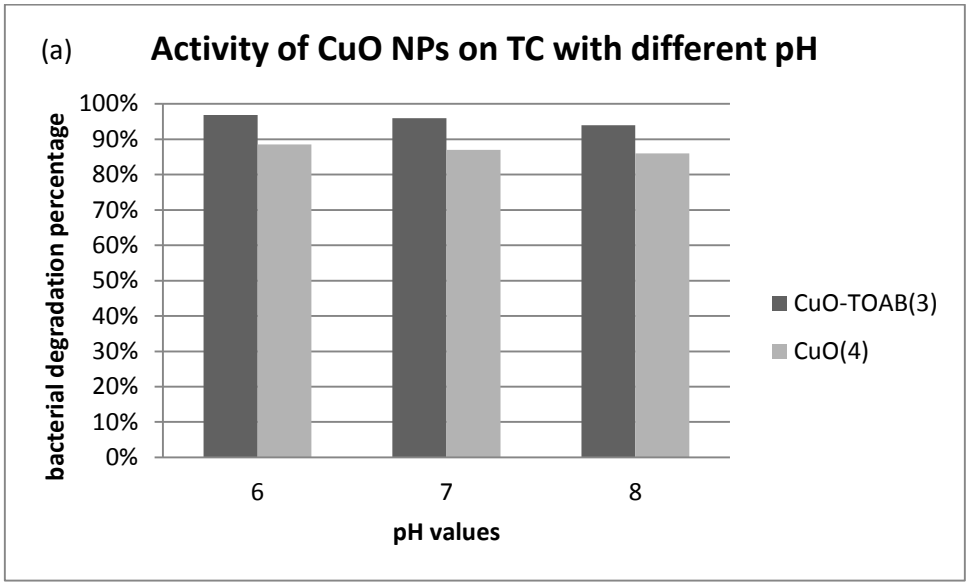


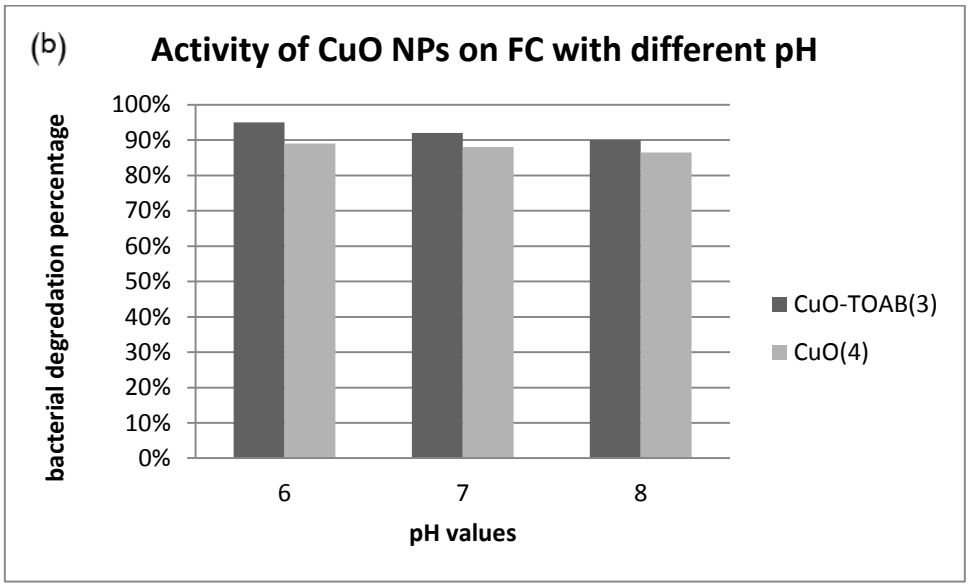
Fig 3.20: The effect of wastewater temperature (15, 25 and 35 °C) on degradation percentage of *E. faecalis* using a) CuO-TOAB(3) and b) CuO(4)

3.2.5 pH effect

Different pH values were investigated to determine the optimal pH for antibacterial activity of CuO NPs with and without TOAB. Phosphate-citrate buffer was used to prepare pH values of 6, 7 and 8 (acidic, neutral and basic media) that represent the optimal range for bacterial growth and were used as test medium for the bacterial degradation percentage.

CuO non-stabilized NPs (10^3 µg/mL) and CuO-TOAB stabilized NPs (10^2 µg/mL) at different pH medium showed slight difference in the TC, FC, *E. faecalis* indicators (Fig 3.21(a) to (c) and Table 3.11).


(a)
Activity of CuO NPs on TC with different pH
bacterial degradation percentage
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
6
7
8
pH values
CuO-TOAB(3)
CuO(4)


(b)
Activity of CuO NPs on FC with different pH
bacterial degredation percentage
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
6
7
8
pH values
CuO-TOAB(3)
CuO(4)

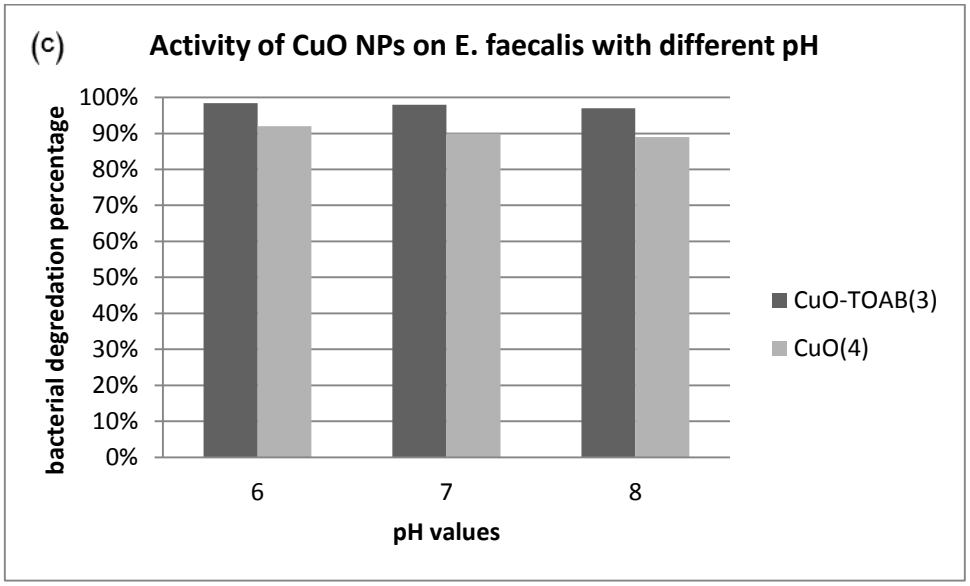


Fig 3.21: The effect of different pH (6, 7 and 8) of CuO(4) and CuO-TOAB(3) on a) TC b) FC c) *E. faecalis* bacteria

The antibacterial activity properties of CuO NPs with and without TOAB surfactant showed increasing degradation percentage of all studied indicators with decreasing pH values with 5% maximum effect (Fig 3.21 and Table 3.11). The slight difference in the bacterial degradation of different pH, exclude the pH effect as an important factor in controlling CuO NPs antibacterial activity. Even though, the maximum effect was seen at pH 6 and 7 that where the pH of the wastewater fell to 6.8, that mean no any further pH treatment is needed for the wastewater.

Table 3.11: Bacterial inhibition growth rate by different pH using CuO(4) NPs (10^3 µg/mL) **and CuO-TOAB(3) NPs** (10^2 µg/mL)

pH \ bacteria	6		7		8	
	CuO(4)	CuO-TOAB(3)	CuO(4)	CuO-TOAB(3)	CuO(4)	CuO-TOAB(3)
TC	88%	97%	87%	96%	86%	94%
FC	89%	95%	88%	92%	86%	90%
E. faecalis	92%	98.5%	91%	98%	89%	97%

3.2.6 Shaking effect on the antibacterial activity

The bacterial inhibition growth rate using CuO(4) after 2 h without shaking was 58%, 51% and 65% for TC, FC and *E. faecalis*, respectively. While the bacterial degradation percent by CuO-TOAB(3) was 90%, 87% and 88% for TC, FC and *E. faecalis*, respectively (Table 3.12). Even antibacterial activity without shaking was prominent; there was a significant increase in the antibacterial activity when used with shaking for CuO NPs without stabilization, while there is much smaller shaking effect for CuO NPs stabilized with TOAB,

In conclusion, shaking may gave a higher chance for bacterial contact effect with the CuO NPs. Higher antibacterial activity noticed with shaking lead us to build the filtration system as a model for wastewater treatment plan.

Table 3.12: Antibacterial activity of CuO(4) and CuO-TOAB(3) on TC, FC and *E. faecalis* with and without shaking

disinfectant	CuO(4) (10^3 µg/mL)		CuO-TOAB(3) (10^2 µg/mL)	
	Without shaking	With shaking	Without shaking	With shaking
TC	58%	82%	90%	96%
FC	51%	76%	87%	92%
E. faecalis	65%	85%	88%	98%

3.3 Flow up test

Flow up test at constant flow rate of 10 mL/min was applied to investigate the antibacterial activity of CuO NPs with and without TOAB surfactant using the optimum parameters investigated through this study including, size, pH and temperature.

The bacterial degradation percentage results were 100% for all used bacterial indicators when the wastewater sample passed through CuO-TOAB(3) stabilized NPs layer. However, when the wastewater sample passed through CuO(4) non-stabilized NPs layer; TC, FC and *E. faecalis* bacterial degradation percentage were 85, 78 and 87%, respectively.

The above results are consistent with the investigated criteria of CuO NPs stabilized with TOAB is more effective in bacterial degradation than that shown in CuO NPs without stabilization. As CuO-TOAB(3) showed complete destruction of all bacterial indicators prove its applicability as a novel wastewater bacterial disinfection technique.

Conclusions

1- Copper oxide nanoparticles (CuO NPs) were successfully prepared by fast, inexpensive and simple quick precipitation method.

2- The NPs size was easily controlled with different temperature; 65, 75 and 85 ºC. However, the increasing in the temperature lead to a decrease of nanoparticles size

3- The sizes of CuO-TOAB stabilized NPs are smaller than CuO non-stabilized NPs that prepared at the same temperature.

4- Rody-stick shape was recorded for all CuO NPs with and without tetraoctylammonium bromide (TOAB) surfactant samples, but it looks more regular for CuO NPs with surfactant, and the same shape for all that prepared at different temperature; 65, 75 and 85 ºC.

5- X-Ray diffractogram peaks showed that all obtained CuO NPs have monoclinic structure.

6- CuO-TOAB Stabilized NPs showed higher antibacterial activity against wastewater bacterial indicators, TC, FC and *E. faecalis*.

7- Bacterial degradation were consistent for the used bacterial degradation indicators; TC, FC and *E. faecalis* and all were less than 100 and 1000 µg/mL for CuO NPs with and without TOAB surfactant, respectively.

8- The results showed that the highest antibacterial activity was for the medium size for both CuO-TOAB stabilized NPs (9.9 nm) and CuO

non-stabilized NPs (11.4 nm), in comparison to other CuO NPs sizes that was found to be 11.5, 9.9 and 7.8 nm for CuO-TOAB stabilized NPs, also was found to be 12.4, 11.4 and 9.1 nm for CuO non-stabilized NPs.

9- Logically, increasing contact time has increased the antibacterial activity of both CuO NPs with and without TOAB surfactant. As the difference in contact time antibacterial effect was small for both CuO NPs with and without TOAB, 2 h incubation time was chosen through this study.

10- The maximum bacterial degradation percentage was seen in CuO NPs without TOAB surfactant stabilization at 35 ºC, while it was at 25 ºC for CuO NPs with TOAB surfactant.

11- With different pH values of wastewater, the antibacterial activity of CuO NPs with and without TOAB surfactant showed slightly effect. However, its activity increases with decreasing the wastewater pH values.

12- CuO NPs with and without TOAB surfactant revealed antibacterial activity even without nanoparticles shaking. However, the antibacterial activity was 70 and 90% for CuO NPs without and with TOAB surfactant, respectively, in comparison to antibacterial activity with shaking that gave a higher chance for bacteria to contact with the CuO NPs.

13- Antibacterial activity of CuO NPs with and without TOAB was found to have higher activity against gram positive bacteria; *E. faecalis*, more than the activity against gram negative; TC and FC indicators.

14- Flow up test through CuO NPs with and without TOAB surfactant layer showed the complete bacterial degradation percentage of CuO-TOAB stabilized in comparison to 78-87% bacterial degradation of CuO non-stabilized NPs for all tested indictors bacteria.

Suggestions for future work

1- Studying the effect of different concentrations of NaOH reducing agent and TOAB surfactant on the CuO NPs size and morphology.

2- Studying the antibacterial activity of CuO NPs with other surfactants.

3- Stabilizing other nanoparticles with TOAB surfactant and studying its antibacterial activity.

4- Studying the activity of CuO NPs against other types of bacteria and pathogens.

5- Applying CuO NPs with and without TOAB surfactant on different water pollutants such as nitrite, nitrate, chloride and other pollutants.

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كلية الدراسات العليا

جامعة النجاح الوطنية

تعقيم المياه العادمة من الميكروبات باستخدام اكسيد النحاس المُصَنَّع بحجم النانو والمغلف بالمذيب السطحي

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قدمت هذه الأطروحة استكمالاً لمتطلبات درجة الماجستير في الكيمياء بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين

2013

تعقيم المياه العادمة من الميكروبات باستخدام اكسيد النحاس المُصَنَّع بحجم النانو والمغلف بالمذيب السطحي

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الملخص

حبيبات النانو يمكن ان تعرف بانها تجمع صغير لذرات عنصر ما، بحيث يشكل مجموع هذه الذرات جزيء يقل حجمه عن 100 نانو متر. تتميز هذه الجزيئات بانها تملك خصائص فيزيائية وكيميائية مختلفة عن الخصائص التي تملكها في حال كانت جزيئات كبيرة، وذلك بسبب الزيادة الكبيرة في مساحة سطحها بالمقارنة مع مساحة سطح الجزيئات الكبيرة.

من العناصر المشهورة في هذا المجال هي الفضة والذهب واكسيد الزنك وكذلك اكسيد النحاس. يمكن تحضير جزيئات بحجم النانو من اكسيد النحاس بعدة طرق منها طريقة ترسيب اكسيد النحاس عن طريق اختزالها باستخدام عامل مختزل.هناك العديد من التطبيقات التي يدخل فيها اكسيد النحاس بحجم النانو منها استخدامها في خلايا الوقود والتطبيقات الطبية وكذلك في تعقيم المياه من البكتيريا.

في هذه الدراسة تم تحضير اكسيد النحاس بحجم النانو بالشكل العصوي وبثلاثة احجام مختلفة واستخدامها كمضاد لثلاثة انواع من البكتيريا في المياه العادمة، وهذه الانواع هي TC و FC و *E. faecalis*، وتعتبر هذه الانواع كمؤشر لوجود انواع اخرى من الملوثات في المياه. وقد تم تحضير اكسيد النحاس بحجم النانو بطريقة ترسيب اكسيد النحاس باستخدام عامل مختزل عند دراجات حرارة مختلفة هي 65 و 75 و85 س وكذلك تم تغليف اكسيد النحاس باستخدام مثبت سطحي له خاصية مضادة للبكتيريا. وقد تم تشخيص اكسيد النحاس بحجم النانو لمعرفة حجم الجزيئات وشكلها باختلاف

درجة حرارة التحضير وتأثير وجود المثبت على الحجم والشكل، وقد تم هذا التشخيص باستخدام اجهزة SEM و XRD .

تم دراسة اثر العديد من المتغيرات على تأثير اكسيد النحاس بحجم النانو على البكتيريا الموجودة في المياه العادمة، وكانت هذه المتغيرات هي حجم جزيئات النانو بوجود وعدم وجود المثبت وتركيز جزيئات النانو ووقت المعالجة وتغيير درجة حموضة المياه العادمة ودرجة حرارتها وكذلك اثر تحريك جزيئات النانو في عينات المياه العادمة على فعاليتها في مكافحة البكتيريا.

اظهرت النتائج ان حجم جزيئات النانو المُحَضَّرة كانت تتراوح بين 7 و 12 نانومتر، بحيث كانت العلاقة عكسية مع ارتفاع درجة حرارة التحضير بحيث كانت كلما ازدادت درجة حرارة التحضير كانت جزيئات النانو بحجم اصغر. وكذلك كانت العينات المثبتة اصغر حجما من العينات غير المثبة عند نفس درجات الحرارة. وكذلك فان جميع العينات المُحَضَّرة كانت بالشكل العصوي.

اظهرت الدراسة فعالية قوية لاكسيد النحاس بحجم النانو المغلف بالمواد الفعالة سطحيا ضد البكتيريا المستهدفة في المياه العادمة، وكذلك كان هناك تأثير للعديد من المتغيرات على فعالية هذه الجزيئات حيث كانت الفعالية الاقوى للجزيئات ذات الحجم المتوسط للعينات المثبتة (9.9 نانومتر) و العينات غير المثبتة (11.4 نانومتر). وكذلك فان فعالية العينات المثبتة كانت اكبر من العينات غير المثبتة وكان هناك تأثير خفيف لزيادة الوقت وتغيير درجة الحموضة بحيث كانت الفعالية تزيد كلما زاد وقت المعالجة ومع انخفاض درجة الحموضة. وكانت اقوى فعالية للجزيئات المثبتة عند درجة حرارة 25 س بينما كانت 35 س للجزيئات غير المثبة.

الفحص على عينات مياه عادمة من خلال تمريره في اكسيد النحاس المثبت اثبتت فعالية تمكن من استخدامها في التطبيقات العملية لتنقية المياه العادمة.