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Evaluating of the Action of Microbial of Nano-Silver Coated Polyvinyl Chloride (PVC) Pipes for Drinking Water

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَيُطَافُ عَلَيْهِمْ بِآيَةٍ مِنْ فِضَّةٍ وَأَكْوَابٍ

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I certify that this thesis "**Evaluating of the Action of Microbial of Nano-Silver Coated Polyvinyl Chloride (PVC) Pipes for Drinking Water**" and submitted by the student (**Zena Hussein ALI**) was prepared under my supervision at the Department of Environmental Engineer / College of Engineering / university of Babylon as a part of requirements for a master's degree of science in Environmental Engineering.

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Dedication

To

*To those who taught me that nothing is impossible in life with
the power of faith and proper planning, to the source of
giving, warmth, and safety*

To my beloved [father and mother](#)

To [my dear husband](#), who gives me encouragement and support

To my children and the light of my eyes

[Ali, Amani, Ridha and Yahiya](#)

To [my sisters](#) who gave me help, and advice

*To [my friends](#) for their unlimited care, support, and
encouragement for me.*

Without whom I could not complete my thesis.

*To [Everyone](#) I had learned from them in my life, I dedicate this
effort with all respect, I wish this to be accepted.*

[Zena](#)

2023

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*"In the name of **ALLAH** the most gracious, the most merciful"*

*It may not be enough to contain the words of thanksgiving...To **ALLAH**...for the strength and hope that keep me believing that this work would be possible.*

*I would like to express my deepest gratitude and respect to my thesis supervisor **Prof. Dr. Alaa Hussein Al-fatlawi**.*

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Abstract

Water pollution by physical, chemical and biologicals' factors is an inevitable phenomenon that requires a certain technology to control such pollution. Drinking water passing through the water distribution network may be at risk of contamination due to pipes crack, repair, pipes connection or construction. In this study, a recent modern methods was used, that is the coating of municipal drinking water pipes partially with silver nanoparticles (AgNPs).

AgNPs were prepared by the green synthesis methods from the aqueous extract of leaves of the *Azadirachta indica* plant. AgNPs were characterized to ascertain the nature of the generated particles by the Ultraviolet–Visible (UV-Vis) spectrum, Fourier Transform Infrared (FTIR) spectrum, X-Ray Diffraction (XRD), Field Emission–Scanning Electron Microscopy (FE-SEM) and Transmission Electron Microscopy (TEM). Four PVC water pipes (3 m in length, 2.54 cm in diameter) were partitioned each into lengths of 30 and 50 cm. Half number of the pipes were coated with silver nanoparticles using the hot air current annealing methods and characterized by using FE-SEM and XRD techniques. Four experimental setup systems were configured to simulate the methods of pumping water through traditional distribution systems.

The antibacterial activity against *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) was tested. In the case of *E. coli* testing, when the water was passed through the pipes of 30 cm, the inhibition rate reached 100% with a concentration of 125 ppm after 15 minutes, while at concentration of 250 ppm, the inhibition rate reached 100% after 10 minutes. In the case of a 50 cm pipe length, the inhibition rate reached 100% at a concentration of 125 ppm at 20 minutes, while at a concentration of 250 ppm, the inhibition rate reached 100% after 15 minutes. As for *P. aeruginosa*, when the water was passed through the pipes of 30 cm, the inhibition rate reached

99.65% with a concentration of 125 ppm after 20 minutes, and 100% at a concentration of 250 ppm after 10 minutes. Also when the length is 50 cm, the inhibition rate reached 98.69% at 125 ppm after 20 minutes, while at 250 ppm, the inhibition rate reached 100% after 15 minutes.

The results of silver content in water passing through the coated pipes measured by using Atomic Absorption Spectroscopy (AAS) complied with the EPA (822-F18-001) and WHO (FWC-WSH-15.02) standards.

The cytotoxicity effect of AgNPs on white blood cells (WBCs) was tested, and the obtained results have shown that there were no significant differences ($P \leq 0.05$) between the tested and control group at the different exposure times with both types of exposure manners, when the inhibition rate was calculated, the highest value was 2.68% in AgNPs concentration of 250 ppm in the case of direct exposure.

From the results of the hemocompatibility test of AgNPs with red blood cells (RBCs) at different concentrations of AgNPs and different times, it was observed that AgNPs did not cause significant ($P \leq 0.05$) hemolysis in the exposed blood samples where the highest hemolysis rate was 1.78% in AgNPs concentration of 250 ppm at both types of exposure without significant differences.

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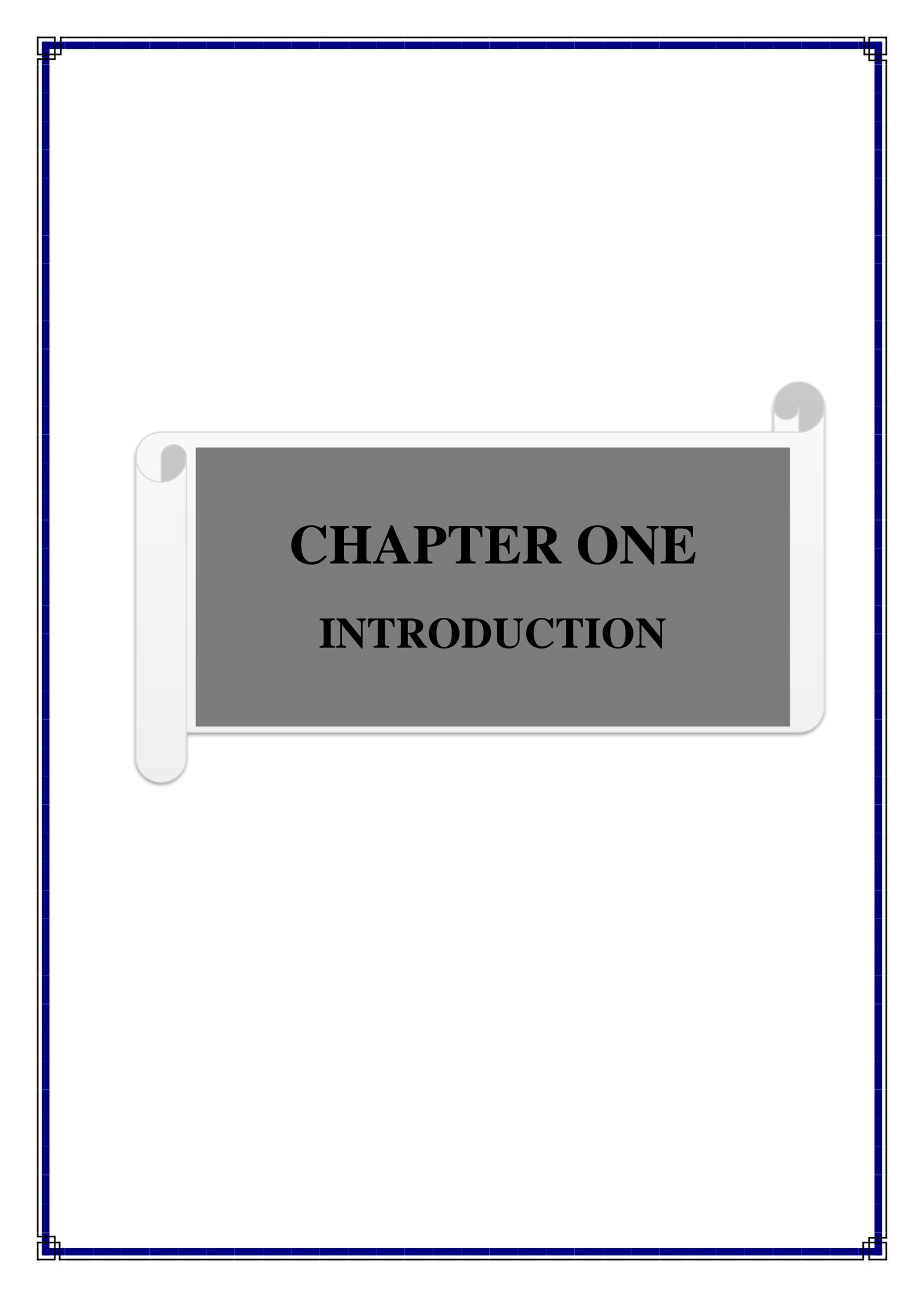
List of Abbreviations

Abbreviations	Description
UV	Ultra-Violet
AgNPs	Silver Nanoparticles
DBPs	Disinfection By-Products
NMs	Nanomaterials
PVC	Polyvinyl Chloride
THMs	Trihalomethanes
WIPO	World Intellectual Property Organization
PCT	The Patent Cooperation Treaty
<i>E.coli</i>	<i>Escherichia coli</i>
B.C.	Before the birth of Christ
<i>G.lamblia</i>	<i>Giardia lamblia</i>
ATP	Adenosine Triphosphate
TDS	Total Dissolved Solids
PH	Hydrogen Ion concentration
EC	Electrical Conductivity
MH	Magnesium Hardness
TH	Total Hardness
DOM	Dissolved Organic Matter
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
UV–Vis	Ultraviolet–Visible
SPR	Surface Plasmon Resonance
FTIR	Fourier Transform Infrared
XRD	X-Ray Diffraction
MH	Muller Hinton
Th	Theta
FESEM	Field Emission Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
AAS	Atomic Absorption Spectrometry
EHT	Event Horizon Telescope
UTIs	Urinary Tract Infections
FCC	Face-Centered Cubic
LSD	Least Significant Differences
Pos	Position
Cts	Counts
FWHM	Full-Width High Maximum
ATP	Adenosine Triphosphate
DNA	Deoxyribonucleic Acid
BIB	Brain Infusion Broth
TVC	Total Viable Count

CFU	Colony-Forming Unit
RPMI	Roswell Park Memorial Institute
WBCs	White Blood Cells
RBCs	Red Blood Cells
EDTA	Ethylene diamine tetra acetic acid
HR	Hemolysis rate
SD	Standard Deviation
LSD	Least Significant Differences
OD	Optical Density

List of symbols

Abbreviations	Description
microns	1×10^{-6} meter
NTU	Nephelometric Turbidity Unit
nm	Nanometer
°C	Degree Celsius
mg/L	Milligram per Liter
gm	Gram
rpm	Revolutions per minute
W	Watt
μL	Microliters
CFU/mL	Colony-forming units per milliliter
S	Sulfate
Cl	Chlorine
O ₃	Ozone
I	Iodine
Br	Bromine
Fe	Iron
S	Sulfate
Mn	Manganese
F	Fluoride
P	Phosphorus
Ca	Calcium
%	Percent
-Sh	Sulfhydryl
PO ₄ ⁻³	Phosphates
NO ₃ ⁻	Nitrate
KMnO ₄	Potassium Permanganate
NH ₄ ⁺	Ammonium ion
H ₂ S	Hydrogen sulfide
PBS	phosphate buffer saline



CHAPTER ONE

INTRODUCTION

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Providing clean and safe drinking water is a major task facing authorities worldwide since water may be heavily contaminated by biological, physical, radiological, and chemical pollutants and subsequently presents different health problems (Pandey, 2006) such as physiological disorders (Verbalis, 2003) and various diseases (Nwabor et al., 2016).

Therefore different tools and techniques were invented for water purification, sterilization, and improving water quality such as traditional, appropriate, and household water treatment methods (Chaurasia, and Tiwari, 2016; Geremew et al., 2018). However, improving drinking water quality was developed significantly, and contemporary techniques were introduced in 1911 such as chlorination (Shinde et al., 2021), desalination (Younos, and Tulou, 2005), sedimentation (Paterniani et al., 2010), coagulation, and flocculation (Muruganandam et al., 2017), ozonation (Wei et al., 2017), ultra-violet (Adegbola, and Olaoye, 2012). However, the most recent technique is Nanotechnology which provides innovative solutions for improving drinking water via disinfection, and enhancing water quality (Ajith, and Rajamani 2021) as mainly Silver Nanoparticles (Rus et al., 2017).

1.2 Silver Nanotechnology

Silver nanoparticles have been recently introduced and widely used in various applications such as medicine (Singh et al., 2008) and therapeutic (Wei et al., 2015), cosmetics (Gajbhiye and Sakharwade,

2016), elastomers (Mcafee, 2013), biotechnology (Sarmast and Salehi, 2016), plant protection (Gupta et al., 2018), textile industry (Uday et al., 2016), food packaging (Simbine et al., 2019) and food industry (Camberos et al., 2019), industrial and biomedical applications (Haider and Kang, 2015) and mainly in water disinfection (Deshmukh et al., 2019) and wastewater treatment (Ganguly et al., 2021).

However, Nano- silver was used intensively in the purification of drinking water (Figoli et al., 2017), treatment of domestic wastewater (Bolaños-Benítez et al., 2020), improving water quality (Dhasarathan, et al., 2018) and removing pesticides (Manimegala et al., 2011). Also, it was used for the removal of various water contaminants such as halide which is a binary compound of a halogen with another element or group (Polo et al., 2017), organic dyes (Khatoon et al., 2018), microbic communities (Zhang et al., 2016), iodide (I^-) (Tauanov and Inglezakis, 2019), ammonium (NH_4^+) content (Chu et al., 2018), and hydrogen sulfide (H_2S) (Shin et al., 2009). In addition to the removal of inorganic odorous compounds (Shin et al., 2008) and certain heavy metal from Different Water Resources (El Awady et al., 2021).

1.3 Municipal Drinking Water Distribution Pipes

Drinking water distribution pipes often reach every home within any particular area to supply them with safe and clean water that is subsequently available all the time without any cutout and sufficient enough to reach upper storing tanks (Khasraw et al., 2021). Obviously, municipal water is consumed for drinking, bathing, washing, and other home needs.

Initially, drinking water distributing network pipes used cast iron, galvanized iron, steel, cooper, polyethylene, asbestos and concrete pipes (Wei, 2021) and as the time passes away, it was found that some types of

these pipes had certain disadvantages that may form health risks (Hossain et al., 2015) such as asbestos-coated pipelines (Di Ciaula and Gennaro, 2016), copper (Oskarsson and Norrgren, 1998) in addition to other problems such as corrosion of galvanized metal pipes (Chawla et al., 2012) and cracking of concrete pipes (Zhang et al., 2020).

1.4 Statement of the Problem

The water problem is one of the most important problems that most countries of the world suffer from, especially developing countries, as a result of the continuous increase in population and the consequent increase in the rate of human consumption of water. Misuse of water, the continuous waste of pure water networks, breakage and wear, in addition to the constant power outages, water can affect the amount of drinkable water. All the points previously mentioned are among the problems faced by many countries in the world, including Iraq, to obtain drinking water [Ahmed and Altaif, 2020], where water pollution with physical, chemical and biological factors is an inevitable phenomenon that requires a specific technique to control this pollution, which leads to purification and disinfection of water. drinking and improve its quality.

Various methods and techniques have been applied to treat drinking water and disinfect it from various contaminants [McFee et al., 2008] in order to provide safe and pure water. Certainly, disinfection of drinking water has effectively prevented water-borne diseases, but the unintended consequences are the generation of disinfection by-products (DBPs), that represent a widespread environmental exposure route to carcinogens [Li and Mitch, 2018], therefore is no secret that carcinogenic and potentially mutagenic byproducts and potential pathogens due to the use of conventional disinfectants such as Cl [McDonald and Komulainen, 2005]. Many of the DBPs are bio accumulative, so they can build up in body

tissues. In fact that some bacterial species such as fecal coliform, *Escherichia coli* and *Pseudomonas aeruginosa* show resistance to chlorination, and this process also enhances the ability of species to exchange antibiotic resistance genes [Jin et al., 2020]. So that, researchers are seeking to develop new multifunctional nanomaterials (NMs) to improve disinfection systems as well as remove organic and inorganic contaminants from water, to improve water purification or treatment processes, many different types of nanomaterials or nanoparticles are used in water treatment processes, and a newer approach is the application of silver nanotechnology [Rus et al., 2017]. However, a recent modern methods, which has not yet been practically applied, is coating municipal drinking water pipes with AgNPs because water passing through the water distribution network may be subject to contamination due to pipe repair, pipe connection or construction and, it is possible that water pollution occurs within the pipe systems that carry drinking water as a result of its presence near the sewage lines, which leads to pollution in the water distribution networks.

1.5 Objectives of the Study

The main objectives of this study can be summarized by the following:

1. Preparing AgNPs using the green synthesis methods at different concentrations to be used as an antiseptic coating to enhance drinking water quality.
2. Studying the performance and ability of the AgNPs as antibacterial disinfectants for killing and preventing any water microbial occurrence through the coated pipes that could cause several health disorders in terms of both endemic and epidemic levels such as several species of bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and

determining the cytotoxicity and hemocompatibility of AgNPs on human blood cells.

1.6 An Outline of this Study

To meet the above-mentioned objectives, the present study is divided into the following chapters:

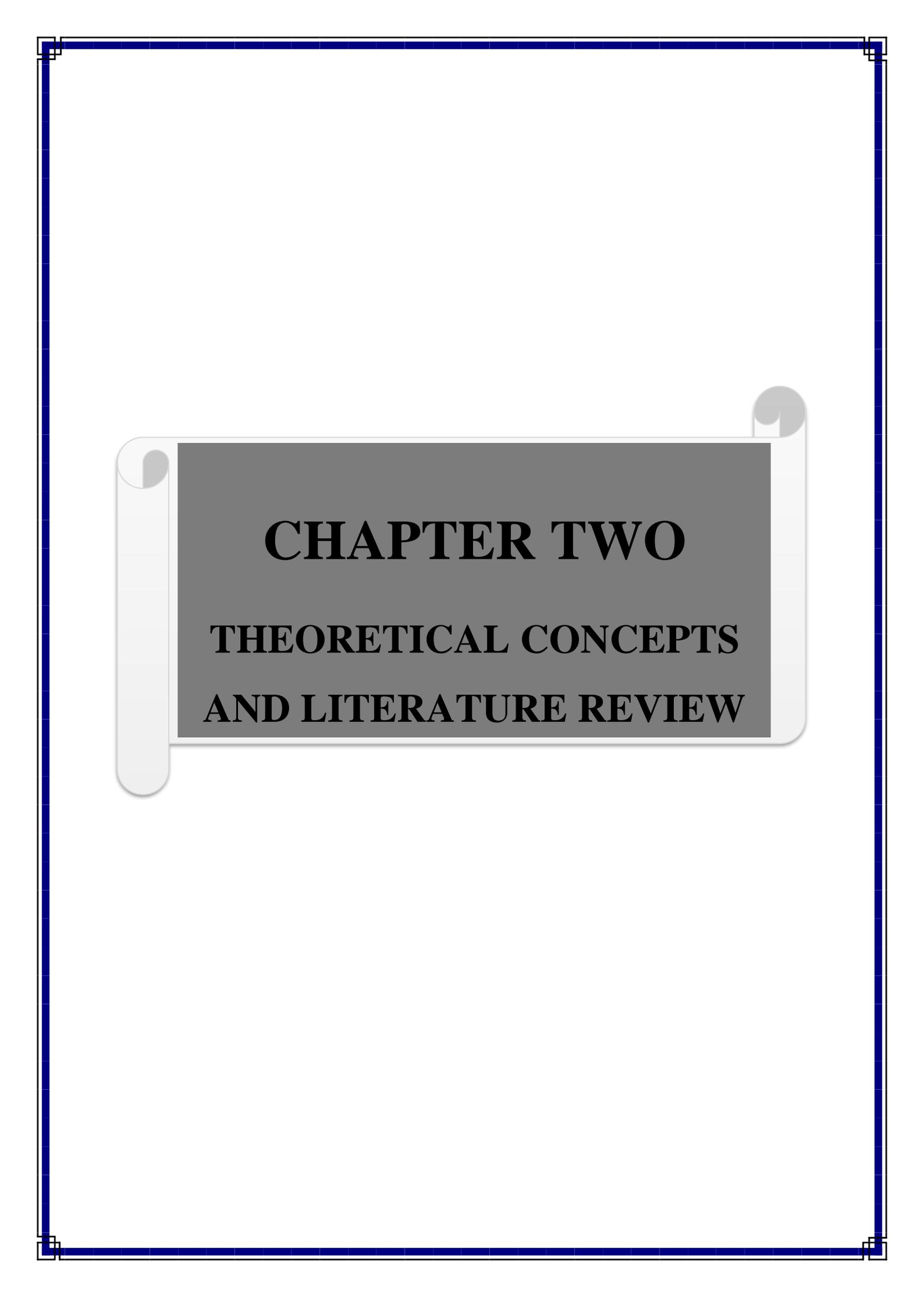
Chapter One: includes a brief introduction to water contamination by biological, chemical, and physical agents. It also covers various techniques of water purification and disinfection in addition to Nano-silver technology.

Chapter Two: gives basic concepts and a literature review of water disinfection and purification methods. In addition, it contains deep detail of silver nano techniques used to sterilize and improve drinking water quality produced by municipal authorities.

Chapter Three: describes materials and methods applied in examining all water variables tested via silver nanoparticles-coated PVC water pipes. Furthermore, it covers statistical analysis.

Chapter Four: displays and discusses the obtained results of experimental works.

Chapter Five: presents the most conclude results and at the same time, it shows probable thoughts that can be recommended for further studies.



CHAPTER TWO

THEORETICAL CONCEPTS

AND LITERATURE REVIEW

CHAPTER TWO

THEORETICAL CONCEPTS AND LITERATURE REVIEW

2.1 Introduction

Obviously Nanotechnology has invaded various life infrastructure fields commencing from furniture industry (Hendy, 2020) to aerospace needs (Edwards et al., 2017) passing by health care (Patel et al., 2015) and beauty products (Effiong et al., 2019), food industry (Rashidi and Khosray, 2011), food packaging (Mylvaganam and Rathnayake, 2020), construction material (Seevakan and Sheeba, 2018) ,road construction (Singh et al., 2019) and water disinfection and treatment such as drinking water (Figoli et al., 2017), wastewater (Abu Shmeis, 2022), groundwater (Ashutosh and Himanshu, 2010), and industrial wastewater (Hasham, 2018).

In fact, nanotechnology concept was initially presented by American physicist Richard Feynman at 1959 quite a long time before it was exercised where he has described the opportunity of scientist to address and handle atoms and molecules but it was firstly used at 1974 by Japanese scientist Norio Taniguchi (Ramsden, 2005) followed by Eric Drexler at 1986 who has used the term nanotechnology in his book (Herges, 2020). However, at 1981 the scanning tunneling microscope was developed to examine individual atoms that helped beginning the modern nanotechnology (Wolf, 2006).

Since these inventions, much attention was focused on silver nanoparticles and much studies were carried out worldwide to examine the efficient disinfection of these particles to control water biological contamination such as bacteria and viruses (Bhardwaj et al., 2021). In addition to improviry water quality in terms of physiochemical standards (Tran et al., 2018). Also, the works have covered other water types such as drinking water (Gadkari et al., 2018), groundwater (Mpenyana-Monyatsi et al., 2012), domestic municipal wastewater (Gagneten et al., 2021), industrial wastewater (Punnoose and Mathew, 2018) and medical wastewater (Perez, 2012).

Furthermore, the published studies have used such silver nanoparticles in coating different water pipes and other components used in water treatment such as various filters (Maharubin et al., 2019) most importantly low-cost materials such as certain plants (Alharbi et al., 2022) and agricultural waste (Sarva and Ahmed, 2015, Vanlalveni et al., 2021).

2.2 Water Disinfection

It is intended to remove, inactivate or kill pathogenic microorganisms, which are destroyed or inactivated, thereby terminating growth and reproduction. When microorganisms are not removed from drinking water, the use of drinking water makes people sick due to the presence of several types of viruses (Dongdem et al., 2009), and mushrooms (Hageskal et al., 2009), and bacteria (Olson et al., 1991) and other water parasites (Hikal, 2020). Table 2.1 illustrates such techniques is nano-silver materials where several international talents which generally used in coating metal and drinking water pipes to act as antibacterial disinfection.

Table 2.1: International Talents of Nano-Silver Coated Drinking Water Pipes.

No.	Invent Title	Invent Number	Invent publication Year	Inventor	Nationality	Language	Current Assignee	Application Field Date
1	Coating for drinking water pipes	DE60102756D1	2005	Jan Robinson	Germany	German	3 M Innovative Properties Co	2001-02-06 by E Wood Ltd
2	Silvernano water pipe	KR20070049450A	2007	Kim Young-i	South Korea	Korean		2005-11-08 by Kim Young-i
3	Nano- silver for coating the iron pipe for the water supply	KR20080070304A	2008	Park Jin-man	South Korea	Korean		2007-01-26 by Ether Bull UPT Co., Ltd.
4	Iron pipe for service water line coated with nano-silver	JP2008215618A	2008	Jin Man Park	Japan	Japanese	KWPT CO. Ltd.	2008-01-25 by KWPT Co. Ltd.
5	Antibacterial nano-silver coating	WO2016043682A1	2016	Ugursoy Olgun Fatih Üstel	WIPO (PCT)	English, French		2015-09-15 by Olgun Ugursoy, Üstel Fatih
6	Nano-silver coating device	CN105983498A	2016	Zhang Limin Wang Qiaofeng Ji Weijun Wu Jiyang Wang Jiayuan	China	Chines	Jiangsu Leezo Biochemistry Technology Co. Ltd.	2015-02-11 by Jiangsu Leezo Biochemistry Technology Co. Ltd.
7	Antibacterial nano-silver coating	EP3194509B1	2019	Ugursoy , Olgun Fatih Üstel	European Patent Office	German, French	Olgun, Ugursoy, Uestel Fatih	2015-09-15 by Uestel Fatih
8	Prepare the methods for the Nano- silver organic-inorganic composite resin of the Nano- silver particles dipping and the antibiotic property water pipe	CN109563274A	2019	Li Huiqing Li Zhengxuan	China	Chines	Local Environment of Corporate Society Worldwide applications	2017-08-09 by the Local Environment of the Corporate Society
9	Nano-silver antibacterial ultrafiltration membrane and preparation methods thereof	CN112169602A	2021	Lin Xiaofeng	China	Chinses	Suzhou Puxi Environmental Protection Technology Co. Ltd.	2020-08-12 by Suzhou Puxi Environmental Protection Technology Co. Ltd.

2.3 Water Disinfection Methods

Generally, clean and safe drinking water is a major task for any municipal authorities to prevent the outbreak of any waterborne disease, so various techniques and methods were applied to achieve this goal.

2.3.1. Water Disinfection by Heat

It is a primitive method, which was practiced quite some time ago where it has been recommended as early as 500 B.C. Furthermore, it works by boiling water to get rid of all biological contamination in drinking water (WHO, 2015).

2.3.2. Water Disinfection by Chlorine (Cl)

It was first used almost in the late of 18 century in England following the outbreak of typhoid disease. Obviously, chlorination was carried out beyond the purification and filtration processes. The disinfection concentrations were relayed on basically the application of a certain chlorine quantity.

Laterally, chlorine gas was used being the least expensive from Cl for water municipal producers (Rossman et al., 1994).

2.3.3. Water Disinfection by Photocatalytic Process.

This methods is solar water disinfection, which means decontamination of water via eliminating, and degrading biological pollutants where it disinfects water by photocatalysis, which increases photoreaction, and in this methods, titanium dioxide is used (Melián et al., 2000).

2.3.4. Water Disinfection by Bromine (Br)

It is alternative methods of drinking water disinfection where it is generally applied as tablets or solutions forms. Br was initially used in

swimming pools and reported that there was no link between asthma and Br. Apparently, Br that used in drinking water is to be scarce of using because it is less effective than chlorine in disinfecting Bacillus spores and as well as DBPs concerns (Kim, 2014).

2.3.5 Water Disinfection by Iodine (I)

It was used as potable water disinfection as early as, the 1900s where it was applied at two concentrations (2.5 and 7.0 mg/L). However, this methods was restricted due to high cost and probable causing toxic DBPs, which is similar to that of other phthalate esters (WHO, 2018).

2.3.6 Water Disinfection by Ozone (O₃)

It was well known as an effective agent and it has been mostly applied in wastewater treatment plants and stagnant water with a high density of microbes. In addition, it is used in bottled drinking water factories to assure biological sterilization. Additionally, it seems that O₃ use may be a rather expensive and not viable option particularly for municipal drinking water (Nghu et al., 2018).

2.3.7 Water Disinfection by Ultra-Violet (UV)

This water disinfection technique is currently applied as being a very effective process for killing microbes waterborne pathogens even those known as chlorine resistance such as E.coli. But, again this methods is not applied in municipal drinking water treatment rather being the most appreciated process in certain cases such as bottled drinking water (Gross et al., 2015).

2.3.8 Water Disinfection by Potassium Permanganate (KMnO₄)

This special methods is used to control and remove iron (Fe) bacteria from the well waters and other drinking water resources where it can eliminate

microbes (Dadhich and Khan, 2014) and chemical water contents such as Fe, manganese (Mn), and H₂S (Khadse et al., 2015).

2.3.9 Water Disinfection by Nano-Silver

It is the most modern and sophisticated technique used in drinking water sterilization and purification (Deshmukh et al., 2019). In addition, it may enhance water quality (Dhasarathan et al., 2018). Despite several international inventions that used silver nanotechnology in the disinfection of drinking water, it is still not applied in municipal drinking water treatment. This may be due to high costs and health fears.

2.4 Nano- Silver Concept

Nano- silver has nanoparticles size between 1 nm and 100 nm. Various forms of nanoparticles may be prepared depending on the desired application. Nevertheless, generally used silver particles are spherical, octagonal, and thin sheets (Khodashenas and Ghorbani, 2019). While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface to bulk silver atoms. Numerous shapes of nanoparticles can be constructed depending on the application at hand. Commonly used silver nanoparticles are spherical, but diamond, octagonal, and thin sheets are also common.

AgNPs are known to have excellent activities as antiviruses, antibacterial and antifungal (Mohammed et al., 2020) and such activities have led to wide applications of AgNPs such as drinking water sterilization (Deshmukh et al., 2019), biomedical and clinical (Naidu et al., 2015), food industry (Camberos el al., 2019) and quite many others.

2.5 Synthesis of Silver Nanoparticles

There are at least three methods for Nano- silver synthesis, which are physical, chemical, and biological (the green) methods. Table 2.2 contains the most popular and used synthesis of AgNPs. Nevertheless, the most applied methods is chemical synthesis which is using various organic and inorganic reducing factors, physiochemical reduction, electrochemical methods, and radiolysis. Obviously, chemical synthesis is an efficient process of chemical reactions for getting a material or more. This occurs via physical and chemical gouging involving one or more reactions (Sertbakan et al., 2022).

It seems clear that the synthetic process of AgNPs may be affected by various environmental factors such as reaction temperature, time, initial hydrogen ion exponential (pH), and concentrations (Singh et al., 2020; Phuong et al., 2020), in addition to biological materials in case of the green synthesis (Singh et al., 2020).

As well, nanoparticles size may play the methods of the green synthesis of silver nanoparticles is considered one of the most important and safest methods for the following reasons:

1. This methods do not include the use of chemical-reducing agents that have poisoning effects if they are present with silver nanoparticles because the application is in contact with human uses (Jasem et al., 2019).
2. The plant extract act as natural weak reducing agents in addition to their work as dispersants that prevent the aggregation of silver nanoparticles and turn them into micro aggregates (Alheety et al., 2019; Salih et al., 2019).
3. The plant extract contain bioactive substances that can inhibit the growth of bacteria, as they remain on the surface of the synthesized silver nanoparticle (Alheety et al., 2022).

Table 2.2: Most used popular physical, chemical and biological methods of silver nanoparticles synthesis.

Type	Methods	References
Physical	Gamma irradiation	Iravani et al., 2014
	Electron irradiation	
	Laser ablation	Iravani et al., 2014
	Ceramic heater	Jung et al., 2006
Chemical	Chemical reduction	Guzmán et., al 2009
	UV-induced synthesis	Huang et al., 2008
	Electrochemical synthesis	Khaydarov et al., 2009
	Electrolysis	Cheon et al., 2011
	Ultrasonic spray pyrolysis	Pingali et al., 2005
	Polysaccharides	Rajput et al., 2017
	Modified Tollens	AbuDalo et al., 2019
	Surfactant	Reddy et al., 2009
Biological (the green)	Fungi extract	Guilger-Casagrande and Lim, 2019
	Bacteria extract	Ali et al., 2018
	Algae extract	Chugh et al., 2021
	Plant extract	Vanlalveni et al., 2021

2.6 Characteristics of Silver Nanoparticles

Nano-silver is characterized, as being highly efficient, quick acting, and hydrophilic. In addition, it is non-poisonous, not allergic, and non-simulating. When it is added, it reduces, the elongation and transmittance of the materials; increases the roughness, and changes the materials' color (Ai et al., 2014).

Furthermore, the Nano-silver is used as an antibacterial disinfectant and here, it unites with the cell wall of pathogenic bacteria and then goes directly inside the bacteria and combine with sulfhydryl (-SH) of the oxygenic metabolic enzyme to deactivate them, to stop inhalation and metabolism in addition to destroying the bacteria (Yin et al., 2020). It is well known that AgNPs possess unique characteristics associated with their small size. All

nanoparticles despite their chemical constituents have a surface area: volume ratios that are extremely high as shown in Table 2.3 (Fortis, 2022). However, several nanoparticles physical properties such as stability and solubility are overriding by the nature of the nanoparticle surface (Jang et al., 2014).

Table 2.3: AgNPs diameter, surface area, volume, and ratio surface area (Fortis, 2022).

Nano sphere Diameter (nm)	Surface area (nm ²)	Volume (nm ³)	Ratio, Surface Area : Volume
10	314	523	0.60
20	1260	4190	0.30
30	2830	14100	0.20
40	5030	33500	0.15
50	7850	65500	0.12
60	11300	113000	0.10
70	15400	180000	0.09
80	20100	268000	0.08
90	25400	382000	0.07
100	31400	523600	0.06

2.7 Silver Nanoparticles Techniques and Applications.

AgNPs technologies and their applications have gained great interest due to AgNPs being one of the most energetic nanomaterials among the many metallic nanoparticles involved in various technologies and applications (Alaqad and Saleh, 2016).

2.7.1 AgNPs Techniques

Nano- silver technique is used in the treatment of potable water, groundwater, municipal wastewater, and all types of industrial wastewater as a disinfectant agent and purification (Figoli et al., 2017).

2.7.1.1 Drinking Water

Following different inventions that used Nano- silver technique as antibiological disinfectants have given, a green and clear route to use such methods in potable water to assure clean and healthy water at least at the lab scale. Therefore, this technique has received great attention worldwide via using metals (Senna et al., 2018), polyethylene (Sadeghnejad et al., 2014), sand filters (Yong-Ho, 2006), ceramic filters (Oyanedel-Craver and Smith, 2008), Kaolin-jute fibers filters (Hussein and AL-Fatlawi, 2020) and microfiltration membranes (Kadhun and Al-Fatlawi, 2022).

2.7.1.2 Municipal Wastewater

Silver nano technology was frequently used in the treatment of municipal wastewater associated with other materials such as cellulose, activated carbons, chitosan, alginate, graphene oxides, titanium dioxides, and silicon dioxide (Ganguly et al., 2021). It was well shown that Nano- silver is more efficient in killing various water bacteria contents (Khaydarov et al., 2019) and capable of eliminating metal ions organic, nutrients, and microorganisms (Ganguly et al., 2021).

2.7.1.3 Various Industrial Wastewater

Scientific attention was focused on the possible application of silver nanomaterials on industrial, medical, and other wastewater to assess Nano-silver efficiency for elimination and removing different wastewater contaminants such as biological (bacteria, viruses, fungus, and other parasites), chemical and physical such as heavy metals content, hydrogen sulfide (H_2S), nitrates (NO_3), sulfate (S), total dissolved solids (TDS), turbidity (Tur), total suspended solidss (TSS), enhance treatment efficiency and minimize treatment costs (Abdelbasir and Shalan, 2019).

2.7.2 AgNPs Applications

Silver nanomaterials can be used in drinking water sterilization, eliminating chemical contents and improving physical water quality.

2.7.2.1 Biological Variable (Bacteria, Fungus and Viruses)

This technique is regarded as an environmental friend because silver nano-particles target bacteria particularly *E. coli* in light and dark conditions where these particles are very active to destroy the bacteria, viruses, microbes (Dhanalakshmi et al., 2013), and microalgae (Pham, 2019). In addition, this technique was used as antiviruses, antifungals (Mohammed et al., 2020), and in general as biocide (Khaydarov et al., 2019).

Obviously, the mechanism of deactivating water biological contaminants by AgNPs is executed in many ways, which represented by the long-term effectiveness, the efficiency at very low concentrations and resulting without, or much fewer wastes via the synthesis process (Bashir et al., 2011).

Interestingly, silver nano-material as a biological sterilizer was examined in different water chemistry conditions and found that silver disinfection was reduced in the case of a divalent cationic solution in comparison with monovalent (Zhang et al., 2012b).

2.7.2.2 Physiochemical Variables

Actually, several works and studies have examined the effect of AgNPs on physiochemical variables such as total dissolved solids (TDS), total suspended solids (TSS), hydrogen ion exponential (pH), total hardness (TH), electrical conductivity (EC), turbidity, calcium hardness (CH) and magnesium hardness (MH) of sewage water as well as the watercolor (Karthika et al., 2019). In addition, other studies have focused on the role of AgNPs on water quality (Tran et al., 2018).

2.7.2.3 Chemical Water Content

It is well documented that all waters contain different chemical substances

In fact, these water chemical contents may occur at elevated concentrations that could form serious health risks unless applied water particularly for drinking and home needs should be purified and sterilized (Weerasinghe et al., 2006).

Several purifying and sterilizing methods (mentioned above) were used to supply pure and safe drinking water in addition to every home's needs. From these methods is the application of silver nanotechnology as a coating substance for water purification filters (Figoli et al., 2017).

In general, certain works were carried out by examining the impact of AgNPs on the removal of water-inorganic odorous compounds (Srinivasan et al., 2008), hydrogen sulfide (H₂S) (Shin et al., 2009), phosphorus (P) (Chen, et al., 2012), nutrient (Zhang et al., 2016), ammonium (NH₄⁺) (Chu et al., 2018) and heavy metals (Venditti et al., 2018). In fact, silver-modified hydrophytes were used for the removal of heavy metal ions from different water resources (El Awady et al., 2021).

2.8 Efficacy of AgNPs

AgNPs efficiency as drinking antibacterial disinfectant was evaluated (Xiao et al., 2021) since these AgNPs have been applied in drinking water as disinfecting and decontaminating agents (Dutta et al., 2021) and to help in sterilizing water from bacteria and viruses. In addition, it enhances water quality to ensure water is safe for drinking and all home requirements. Since AgNPs were used in coating water filters and membranes, it was found that these methods are more effective in destroying water *E.coli* and other bacteria species (Zhang et al., 2012a; Khaydarov et al., 2019; Bhardwaj et al., 2021). Interestingly, other studies have reported complete growth inhibition of both

gram-positive and negative bacteria *Staphylococcus aureus* and *E. coli* (Li et al., 2021).

However, such AgNPs performance may be governed by environmental factors such as pH and temperature (Phuong et al., 2020). In addition, it may be affected by water-dissolved organic matter (Zhang et al., 2012a), Ca and S (Deshmukh et al., 2019), and particle size (Dong et al., 2019).

2.9 Risks and Benefits of AgNPs

There are certain fears and concerns initiated by using AgNPs in drinking water sterilization challenges although silver was reported to have strong efficiency as a drinking water disinfectant of various biological contaminants such as bacteria, viruses and fungi (Ferdous and Nemmar, 2020).

Nonetheless, there are several studies have indicated possible toxic health effects targeting the nervous system (Korani, et al., 2015). In the other hand, numerous studies right now have used AgNPs as an antibiological disinfectant in drinking water and did not report major health effects since it was used at an almost very low concentration that was sufficient enough to kill bacterial species such as *E.coli* and other bacteria that occurred in water (Li et al., 2021) and can enhance water quality and remove toxic contaminants (Rashid et al., 2021).

At last, it is well confirmed by the most recent study that the application of silver nanoparticles in drinking water sterilization does not result in releasing silver ions into the water (Vanlalveni et al., 2021).

2.10 Mechanism of Killing Bacteria by Silver Nanoparticles

The mechanism by which bacteria are killed by silver nanoparticles is still not established in a precise and definitive manner, therefore it has not been sufficiently explained (Ramkumar et al., 2017; Li et al., 2019). However, several anti-bacterial mechanisms have been proposed, which are shown in Fig. (2.1). The most important of these points is the release of silver nano-ions after their affinity with bacteria, which will bind coordinately with many active atoms present in the protein structure. So, it can be considered as an effective killing mechanism after changes in cell wall structures, the cytoplasmic membrane in addition by the active components in them. After that, the silver nanoparticles abides to the cytoplasmic membrane, which results in the tearing of the bacteria encased and consequently, retarding respiratory enzymes, which stops the production of Adenosine triphosphate (ATP).

The other mechanism is the involvement of silver nanoparticles with sulfur (S) and phosphorus (P) atoms in the DNA, thus interrupting the DNA replication process. Finally, the silver ions can cause the deformation of the ribosomes and thus radically change the cell components (Meikle et al., 2020).

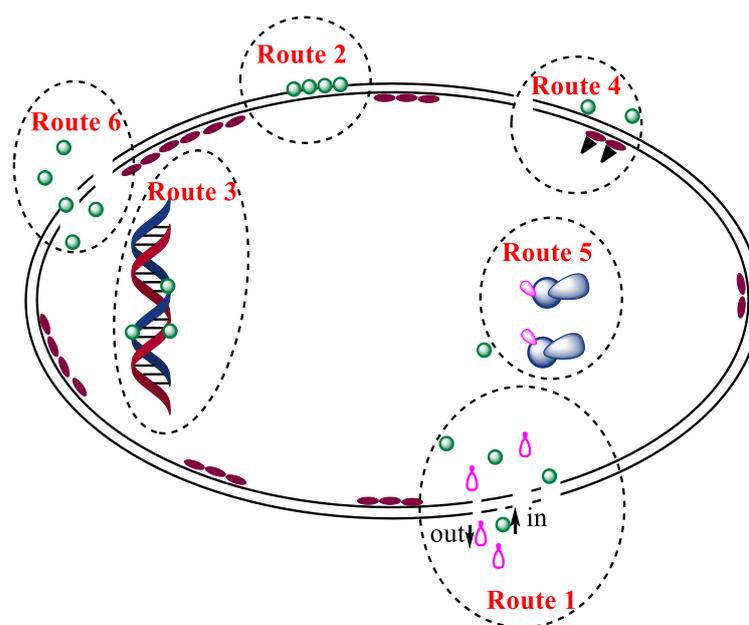


Fig. 2.1: The antibacterial effects of silver nanoparticles, (Yin et al., 2020).

The antibacterial actions of silver nanoparticles (AgNPs):-

Route 1: The release of organelles from the cell after the penetration of bacteria from the ruptured membrane.

Route 2: Changes in the membrane resulting from the accumulation of silver on the surface of the membrane.

Route 3: Prevent DNA replication through the interaction of silver with phosphorus and sulfur.

Route 4: Cell membrane rupture by reactive oxygen species.

Route 5: Negative changes in ribosomes (denaturation) by silver ions released from silver nanoparticles.

Route 6: A defect in the cytoplasmic membrane and the cell wall related to the freed of the silver ions from the silver nanoparticles, which stuck to or pass through the cytoplasmic membrane.

2.11 Bacterial Resistance to Silver

AgNPs were successfully used as disinfectants of drinking water in addition to the possibility of enhancing water quality. In addition, such particles have been applied in the treatment of all municipal, industrial, agricultural, and medical wastewater. Nevertheless, certain studies have mentioned the bacterial ability to develop silver antibiotic resistance (Ellis et al., 2018; Nyika, 2021).

However, it seems that possible evolution of bacterial resistance is not yet fully documented, but gram-negative bacteria *E. coli* and *P. aeruginosa* seem to be capable of evolving tolerance to AgNPs where such case may occur when these bacteria species being frequently subjected to these nanoparticles via producing adhesive flagellum protein that helps in gathering nanoparticles. Nonetheless, such findings may need much attention and deep further investigations since any evolved tolerance process needs genetic background that can assist to select tolerant genes (Nyika, 2021).

2.12 Environmental Costs of AgNPs

The environmental behavior of AgNPs in natural water bodies is still undefined and it needs an urgent examination and searching the fate of these AgNPs. However, a study carried out in Xiamen City, southeast China on saline natural water has shown that such nanoparticles stayed stable in low saline water content (Li et al., 2019). Obviously, adverse environmental impacts of AgNPs are associated with the applied concentration (European, 2013). Previous studies have suggested using low AgNPs concentration to avoid any environmental effects (Hartemann, et al., 2015). Other studies have indicated the actual needs for more investigations for better understanding and predicting proper and clear effects of AgNPs on the environment in terms of tasks, threats, and advantages (McGillicuddy et al., 2017).

However, for proper assessment of environmental impacts due to AgNPs, it is necessary to monitor their behaviors and control any possible effect (Fiorati et al., 2020) while other studies have focused on the possible removing methods of these AgNPs from the environment (Syafiuddin et al., 2019). Prior to occurring any harm effects where the most recent study has suggested to applying a phytoremediation technique used to remove pollutants from soil, air, and water Sources (Ihtisham et al., 2021).

2.13 Growth of Microorganisms in the Distribution Pipes System of Drinking Water

Water is one of the most environmental components that are subject to pollution, because it has special unique properties that make it more capable of receiving different types of pollutants. Biological microbes may break through the drinking water distribution systems where they survive and occasionally bloom in the distribution systems, which increases the possibility of spreading water-borne diseases. Fecal pollution is a main way of microbial

infections. These include bacteria, viruses, and parasites that occur naturally in the intestines of human beings and other warm-blooded animals. Likewise, drinking water inside the pipes of distribution networks is contaminated in the case of broken pipes. The presence of accidental connections and others (US-EPA, 2016).

2.14 Microbial Monitoring in Distribution Systems

Water utilities use specific and standard methods, enshrined in legislation, for microbial quality control and thus water safety within Drinking Water Distribution System (DWDS). Commonly used methods are based on the quantification of microorganisms through heterogeneous platelet counts and indicators of the presence of feces such as coliform and *E. coli*. Culture-based methods are suitable diagnostic tools due to their ease of performance and relatively low cost. However, cultivation methods are time-consuming to get results, and the approach typically accounts for less than 1% of the total diversity in environmental samples (Riesenfeld et al., 2004). In addition, conventional bacterial indicators may fail to capture all potential problems.

Similarly, water-borne pathogens, which are not detected by conventional methods, are increasingly being observed in DWDS due to deteriorating source water quality and changing environmental conditions (Levy et al., 2016).

2.15 Indicator Bacteria (Coliforms)

Coliform bacteria are spread in the environment via the feces of human beings and animals. The coliform bacteria constitute genera that are very familiar with the utilization of lactose to release acid and gas, or have a D-galactosidase enzyme that is capable of employing chromogenic

galactopyranoside substrate for growth (Madappa, 2014). Some types of these bacteria are:

- **Total coliforms**

This term refers to a wide array of gram-negative stained bacteria having almost rod-shaped and share several characteristics. This group includes thermotolerant coliforms and fecal bacteria in addition to certain bacteria that can be insulated from various environmental sources. Detection of coliform bacteria in water indicates potential fecal pollution and thus provides information on treatment efficiency and after-growth (Molelekwa et al., 2014).

- ***Escherichia coli* (*E. coli*)**

Coliform bacteria (*E. coli*) are characterized as gram-negative, facultative anaerobic, and stick-shaped; with several flagella and short, fine appendages (Fimbriae) surrounding the bacterial cell. However, these fecal bacteria are widely found in human beings and different animal intestines. Obviously, coliform bacteria can cause several health disorders that target urinary tract and intestines causing recurrent the urinary tract infections, mild or watery diarrhea, nausea, and vomiting in addition to abdominal pains. As such coliform bacteria are very tiny and cannot be seen unless, a powerful microscope is used. Furthermore, cultivated such bacteria on nutrient agar is required for proper identification under special conditions (Rock and Rivera, 2014).

It seems clear that water *E. coli* content is clear evidence of sewage is currently contaminated by fecal bacteria where such bacteria are commonly originated from human and animal waste. Due to different weather circumstances, *E.coli* bacteria may find their routs in various water bodies and cause water contamination and may end up in drinking water (Bharadwaj and Sharma, 2016).

Major coliform infections caused by such bacteria may be due to ingesting contaminated water resulting in dysentery; urinary tract infections, diarrhea; and vomiting. Meanwhile *E. coli* bacteria can be associated with septic shock to cause fever and hypotension or hypothermia with hypotension. In extreme circumstances, it would be intricate by hepatic failure, uremia, acute respiratory distress syndrome, coma, and death.

It is the systematic reaction to endotoxin (cytokines) or lipopolysaccharide that happens with the bacteria which may lead to deploy blood coagulation process within the vascular to end in death (Madappa, 2014).

2.16 *Pseudomonas aeruginosa* (*P. aeruginosa*)

Pseudomonas genus bacteria especially *P. aeruginosa*, are widely occurred and spanned in various environmental habitats such as soil, water, and organic humus, and frequently responsible for infections in the urinary and respiratory systems, in addition to the skin and occur in the bloodstream, particularly in those of poor immunity patients (Kaye and Pogue, 2015). These bacteria, apparently are well-known as resistant to several antibiotics (Neves et al., 2011). These bacteria have the proper ability to be much better protected from harmful environmental impacts, normal immune thrusters, antimicrobial stuff, dehydration, and radiation (Bjarnsholt, 2013).

P. aeruginosa bacteria were obviously insulated via bacterial tests of several mineral water types such as chlorinated, dechlorinated, and mineral (D'Aguila et al., 2000; Peil et al., 2015), nevertheless, they were not covered in routine monitoring and analyzing of drinking water.

The present work is designed to improve and protect the distribution network of drinking water from pollution with pathogenic types of bacteria, which can be considered the first study regarding Polyvinyl chloride (PVC) pipes used for conveying and distributing municipal drinking water partially coated with AgNPs.

2.17 Gab of knowledge

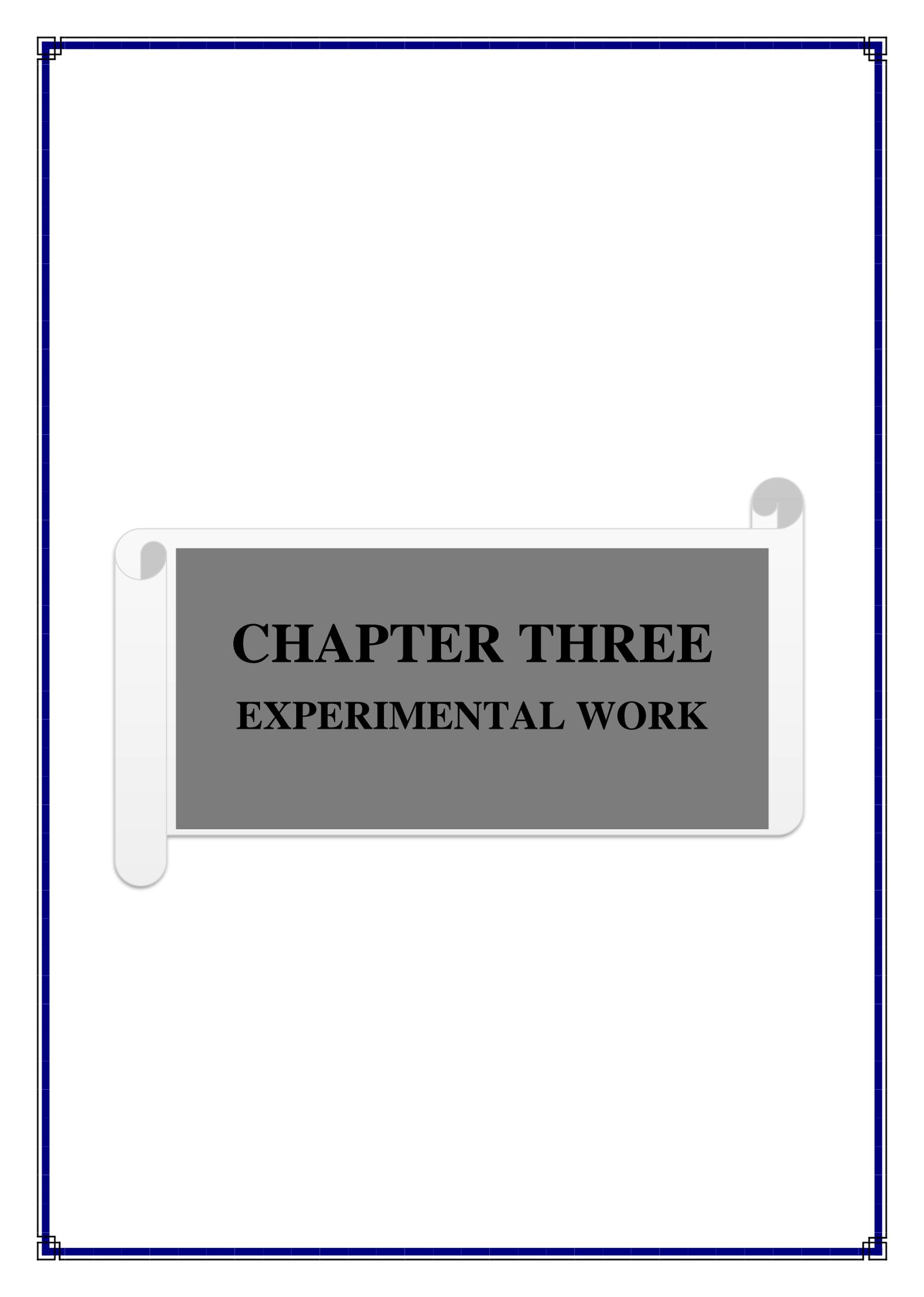
Despite many international patents that claimed applying silver nanoparticles in coating drinking water pipes, but such technique was not applied in terms of municipal drinking water plants worldwide and this may be due to several factors such as high working cost, probable health impacts, stability time of silver coating and it may result in silver tolerance evolution by water bacteria and consequently it will fail in disinfection of water bacterial content of water. Accordingly, these factors may be behind of unavailable researches and works trying such technique and then presenting obvious gap in available knowledge relating to the use of AgNPs as coating materials of municipal drinking water pipes.

2.18. Literature view

Obviously, it seems that there very limited available studies and works related to the subject particularly drinking water PVC and metal pipes and as follows:

- a. In 2011, a study carried out by Heidar pour et al has examined Complete removal of pathogenic bacteria from drinking water using Nano-silver coated cylindrical polypropylene filter where they have tried to investigate the removal of *Escherichia coli* bacteria from drinking water using Nano- silver-coated polypropylene water filter.
- b. In 2013, a Ph. D work entitled “Application of silver nanoparticles in drinking water purification” presented by Zhang, H. who has examined various variables relating to bacterial water disinfection.
- c. In 2014, Tugulea et al., have assessed the impact of Nano-silver in drinking water and drinking water sources: stability and influences on disinfection by-product formation.

- d. In 2015, Praveena and Aris have used low cost materials coated with AgNPs as water filter in *Escherichia coli* removal and shown that these low-cost materials have shown potential efficiency in the removal of *E. coli*, and the silver concentration in the effluent is below the permissible limits.
- e. In 2018, Spoiala et al., have studied the possible way of using AgNPs for water purification.
- f. In 2019, Khaydarov et al carried out a work for applying AgNPs as water bacterial biocides and reported that the antimicrobial activity of silver ions was superior to that of silver nanoparticles.
- g. In 2020, Nagar and Pradeep have tested clean water using AgNPs in terms of needs, gaps, and fulfillment.
- h. In 2021, Vela-Cano et al., have shown that silver antimicrobial coating in metal pipes has prevented microbial biofilms.
- i. Also, in the same year (2021), Bruna et al., have carried out study to examine the effect of AgNPs and their water antibacterial application.



CHAPTER THREE
EXPERIMENTAL WORK

CHAPTER THREE

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3.1 Introduction

This chapter presents the experimental work of the study to determine the performance of coated water pipes in terms of disinfection in addition to assessing the affecting behavior of AgNPs over time. It is consisting of four parts:

The first part includes the preparation of AgNPs from the aqueous extract of the leaves of the *Azadirachta indica* plant and the characterization of synthesized AgNPs. The second part is concerned with the water PVC pipes which have been coated by AgNPs at two concentrations with different water pipe lengths, the characterization of water PVC pipes after being coated by AgNPs in addition to the design of four set of water PVC pipes.

The third part examines the antibacterial impacts of these coated water pipes against *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Also, it includes experiments examining the relationships between certain parameters and removal efficiency. The fourth part covers the testing of several physical and chemical properties of drinking water through its passage by a silver-coated water distribution system, such as hydrogen Ion concentration (pH), total dissolved solids (TDS), and electrical conductivity (EC). Furthermore, it examined drinking water Ag content using Atomic Absorption Spectrophotometer (AAS) after passing through these experimental pipes coated with AgNPs at different times.

Finally, the cytotoxicity of AgNPs was determined by MTT assay and hemocompatibility of AgNPs with human blood cells at different concentrations of AgNPs

3.2. Experimental Work

Fig. 3.1: shows a flow chart of the experimental work.

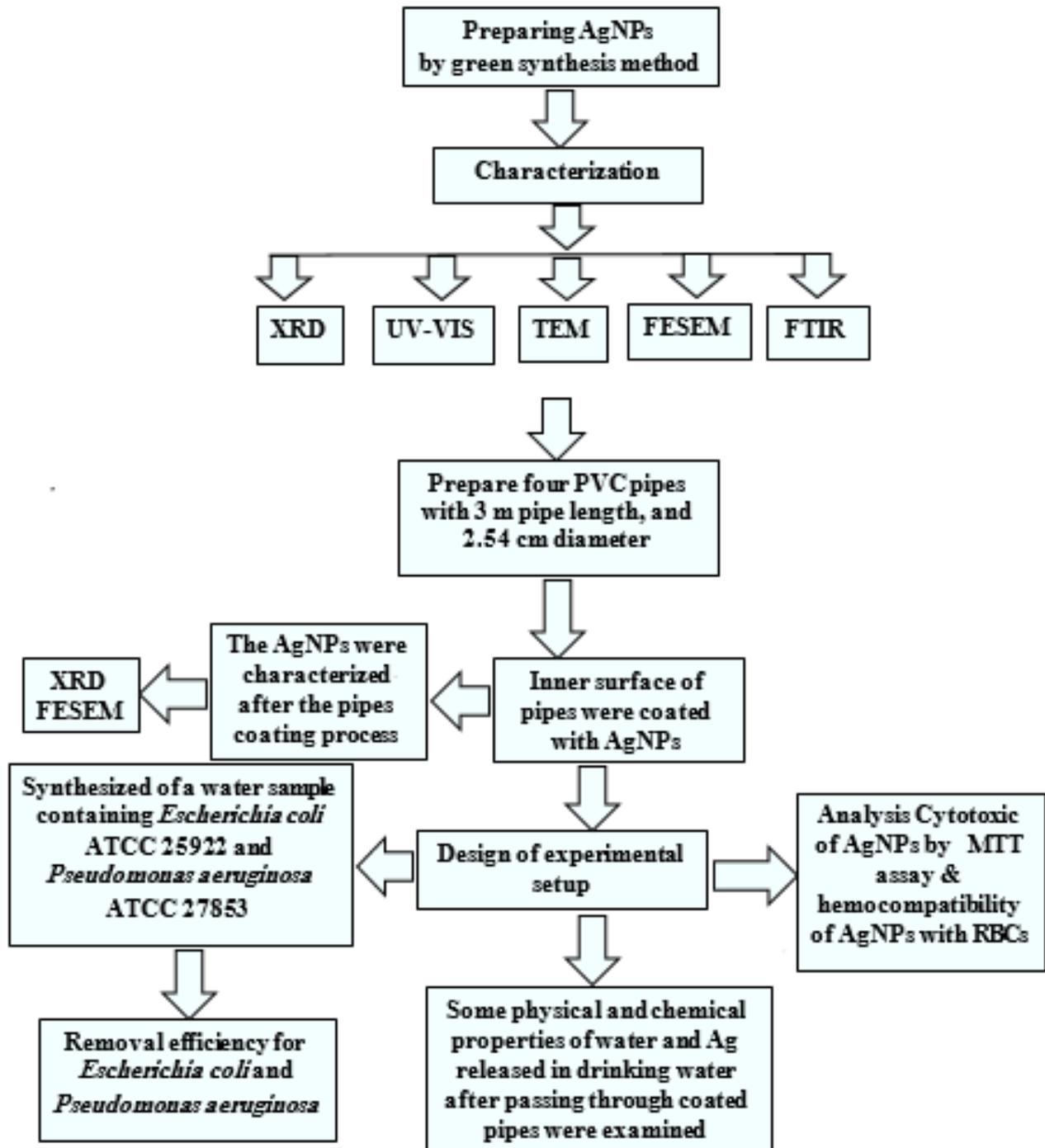


Fig. 3.1: Flow Chart of the experimental work.

3.2.1 Tools and Equipment

The tools and equipment utilized are listed in Table 3.1.

Table 3.1: Tools and equipment used in experimental work.

Instrument	Model	Origin
Atomic Absorption Spectrophotometer (AAS)	UCD-150	China
Autoclave	TR250N	Chania
Culturing Hood	LBC1203B-B2	Korai
Electrical Conductivity(EC)	P1-700PC	Taiwan
Electronic Balance	JA203P	Japan
Elisa Reader	Dana 3200	UK
Field Emission Scanning Electron Microscopy (FESEM)	MIRA111	Czech
FTIR (ATR)	1800	Japan
Heating magnetic stirrer	Velp	Korea
Incubator	LIB-030M	Korea
Microwave	Daewoo	Germany
PH meter Japan	5011A	Japan
Safety cabinet	LBC1203B-B2	Korea
Total dissolved solids,	Lovibond	Germany
Transmission Electron Microscopy(TEM)	Phelps CM10,	Holland
ultrasonic system	UP400S	Germany
UV-VIS spectroscopy	1900	Japan
X-ray diffraction (XRD)	PW1730	Holland

3.3 Bio-Synthesis of Silver Nanoparticles

The aqueous extract of neem leaves was prepared based on the previous study (Roy et al., 2017)

3.3.1 Plant Material

Leaves of *Azadirachta indica* (*Meliaceae*), were collected and identified by the assistant staff of Science College - Baghdad University where all collected leaves have been washed with tap water to remove the dust and impurities, then they were soaked with deionized water. After that, 25 g of washed leaves were mixed with 200 mL of deionized water using an electrical blender to extract the

bioactive compounds. The mixture was heated in an open vessel at 70 °C for 30 min.

After that, the extract was filtered through cotton material to remove the large remaining pieces, and the solution was centrifuged for 10 min at 12000 rpm to obtain a clear solution without any suspended particles as shown in Fig. (3.2).

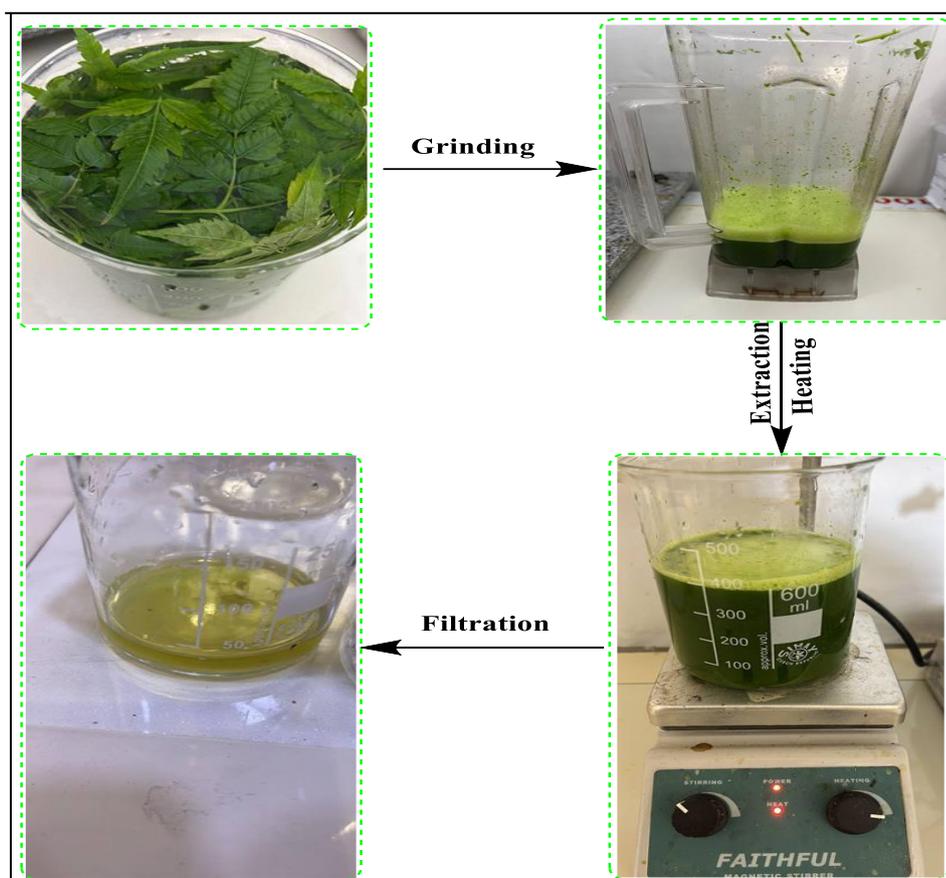


Fig. 3.2: Synthesis steps of leaves extract.

3.3.2 Biological (the green) Synthesis of Silver Nanoparticles

About 0.55 g of silver nitrate (AgNO_3) as presented in Table 3.2 was dissolved in 1000 mL deionized water and heated to 50 °C in a dark container and 5 mL of *Azadirachta indica* leaves extract was wisely dropped into the silver nitrate solution under vigorous stirring at 700 rpm. The color was

changed to yellow within 30 min then; another 5 mL of *Azadirachta indica* leaves extract was added (Roy et al., 2017).

Table 3.2: Properties of AgNO₃, (Lin, 2014).

Property	Description
Chemical Formula	AgNO ₃
Molar mass	169.87 gm/mole
Appearance	colorless crystals
Density	4.35 gm/cm ³ (24 °C) 3.97 gm/cm ³ (210 °C)
Melting point	209.7 °C (409.5 °F; 482.8 K)
Boiling point	440 °C (824 °F; 713 K) (decomposes)
Solubility in water	122 gm/100 mL (0 °C) 170 gm/100 mL (10 °C) 256 gm/100 mL (25 °C) 373 gm/100 mL (40 °C) 912 gm/100 mL (100 °C)
Solubility	Soluble in acetone, ammonia, ether, glycerol

This process was repeated until a red color solution appeared which is the evidence for the formation of nanoparticles. The product was centrifuged at 12000 rpm for 15 min. The collected precipitate was washed thrice with deionized water and hot ethanol to remove unreacted bioactive materials from the plant extract as shown in Fig. 3.3.

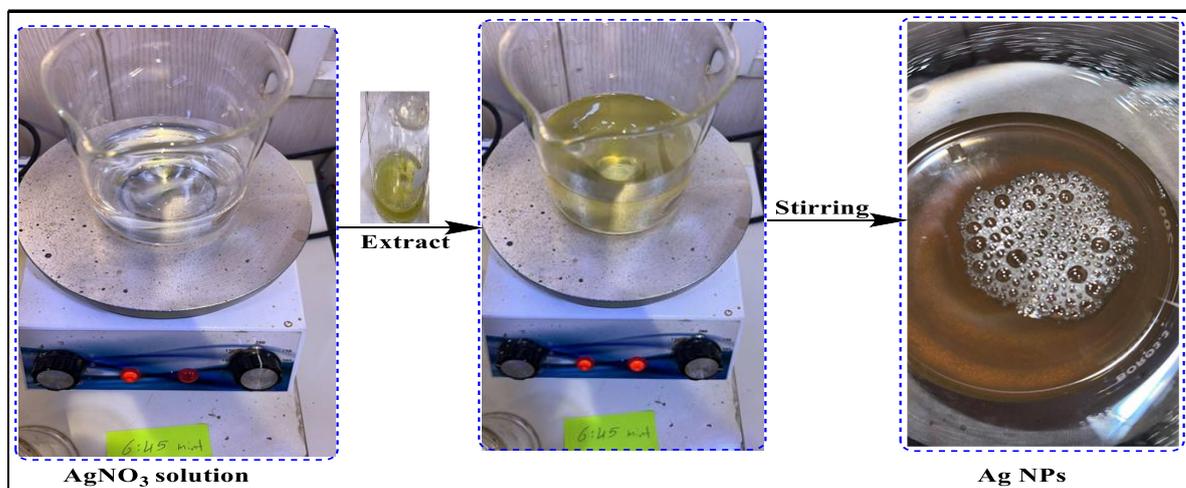


Fig. 3.3: The green synthesis of silver nanoparticles.

3.4 Characterization of AgNPs

3.4.1 Ultraviolet–Visible (UV-Vis) Spectrometer

The prepared solutions were placed under different conditions in a quartz cell with a thickness of (1 cm) at room temperature. The color change of the colloidal solution of the materials used indicates the generation of nanoparticles. The absorption spectra of nanoparticle solutions were measured using a UV-Visible dual beam spectrophotometer within the wavelength range (190-1100 nm) using a (UV-Visible 1900 double beam spectrophotometer, manufactured by Shimadzu Corporation, Japan) at the Physics and Chemistry Department/Nano-science and Technology /Al Zahra University /Iran.

3.4.2 Fourier Transform Infrared (FTIR) Spectrum

FTIR spectrum is widely used technique for the evaluation of industrially manufactured materials and is frequently applied as the first step in the material analysis process where the change in the featured pattern of absorption ranges obviously indicates an alteration in the composition of the material or contamination existence. If any trouble with the product is defined by visual inspection, the origin would be typically determined by FTIR methods. This technique is worth trying for chemical structure analysis of smaller particles which typically range from 10 to 50 microns in addition to larger areas on the surface.

3.4.3 X-Ray Diffraction (XRD)

The powder's XRD technology provides complete information about the type of substance under analysis as well as information about the purity of the compound or mineral as well as its ability to determine crystalline levels and thus determines the crystal system. Acutely, it is intensively applied in chemical examinations in terms of quantity and quality phenomenon especially by electronic microscopes, based on this, X-ray diffraction measurement of the prepared silver nanoparticles was performed, as shown in Fig. (A.1).

3.4.4 Field Emission Scanning Electron Microscopy (FESEM) and Transmission Electron Microscopy (TEM) of AgNPs

Silver nanoparticles are usually characterized by imaging techniques in order to determine the shape and size of nanoparticles. Thusly determining the possibility of using them in the appropriate type of application depending on the surface area resulting from each geometric shape.

FESEM as shown in Fig. A.2 was used to determine the shape and size of nanoparticles, also, TEM technique shown in Fig. A.3 was followed to determine the AgNPs particle size, shape and distribution.

3.5 Coating of PVC pipes with (AgNPs)

3.5.1 Coating of PVC Pipes with 125 ppm of AgNPs

To design the water distribution setup, two PVC water pipes with a length of 3 m and a diameter of 2.54 cm (1 inch) were used. The first pipe was partitioned into 10 parts of 30 cm in length each whilst the second pipe was also partitioned into 6 parts of 50 cm in length. These pipes were washed thoroughly with tap water and distilled water to remove dust ,salts and left to dry by air flow. After that, all dried pipe parts were washed with a thinner to remove all oils and organic contaminants as shown in Fig. (3.4).

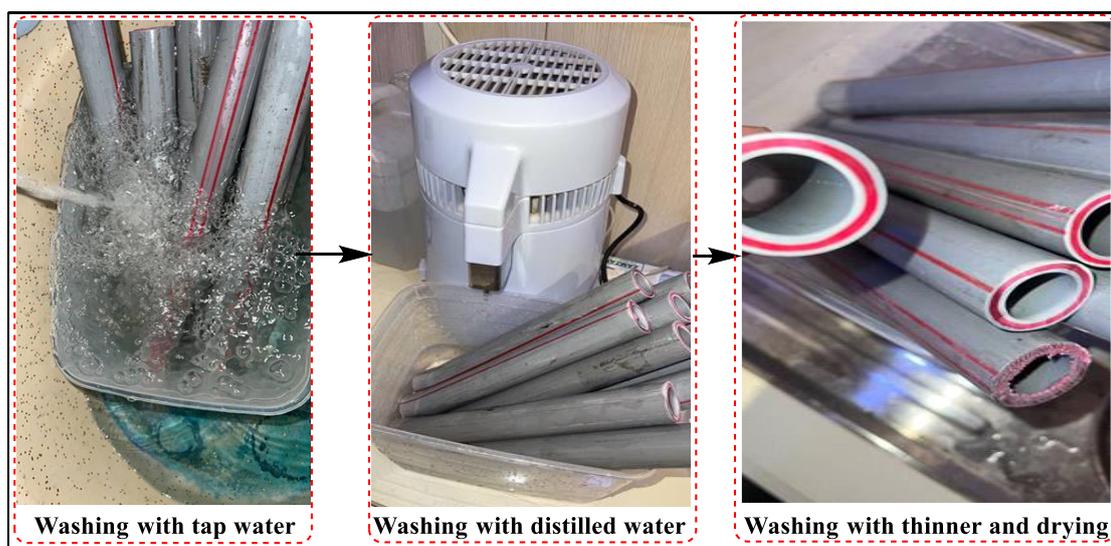


Fig. 3.4: Cleaning steps of pipes.

Silver nanoparticles were homogenized using a 150 W ultrasound probe for 5 min. Furthermore, dried clean pipes were separately immersed in the silver nanoparticles solution (125 ppm) for 10 seconds and left to dry by hot air for 25 seconds.

This process was repeated ten times to insure the complete coating of the inner surface of the PVC pipe with AgNPs. Finally, the experimental silver-treated water pipes were washed with tap water to remove silver nanoparticle residues that did not participate in the coating process, as shown in Fig. (3.5).

The water pipes were coated by AgNPs following the same methods mentioned in section 3.5.1 using concentration of 250 ppm.

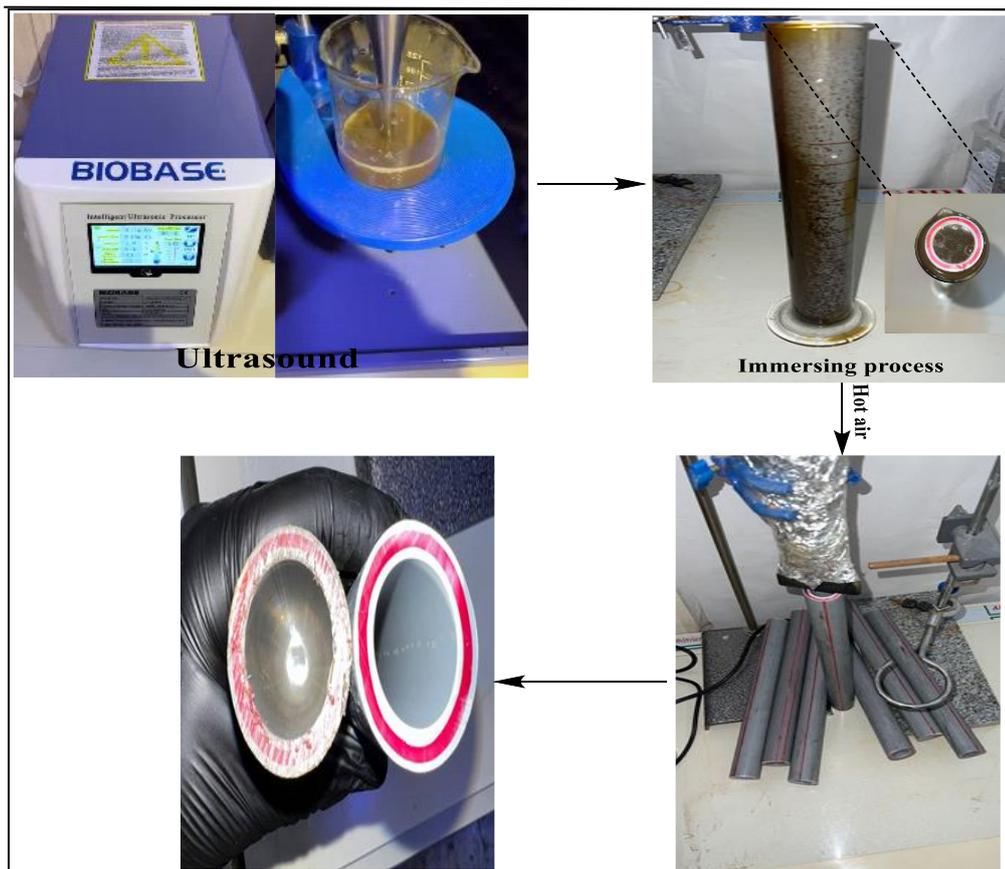


Fig. 3.5: Steps for the preparation of PVC pipes coated with AgNPs.

3.5.2. Characterization of PVC water Pipes Coated with AgNPs

The PVC water pipes were coated with silver nanoparticles following the hot air current annealing methods. Two concentrations of silver nanoparticles (125 ppm and 250 ppm) were applied to determine the sufficient concentration and its relationship with the time factor. These water pipes were characterized using FE-SEM and XRD techniques in order to determine the presence of silver on the surface of the pipes, in addition to assess the differences resulting from the change in concentration.

3.6. Design of Experimental Setup Components

Four experimental setup systems were configured to simulate the methods of pumping water in traditional distribution systems, which were as follows:

1. Design of a PVC pipes setup with a length of (30 cm) coated with silver- nanoparticles (250 ppm) connected alternately with uncoated pipes of the same length.
2. Design of a PVC pipes setup with a length of (30 cm) coated with silver- nanoparticles (250 ppm) connected alternately with uncoated pipes of the same length
3. Design of a PVC pipes setup with a length of (50 cm) coated with silver- nanoparticles (125 ppm) connected alternately with uncoated pipes of the same length.
4. Design of a PVC pipes setup with a length of (50 cm) coated with silver- nanoparticles (250 ppm) connected alternately with uncoated pipes of the same length.

All these multiple divisions of pipes with different concentrations of AgNPs as shown in Figs. 3.6 and 3.7 with their parts and the purpose of each part, pointing out that the pipes are connected in the form of a coated pipe part with an uncoated pipe part and so on.

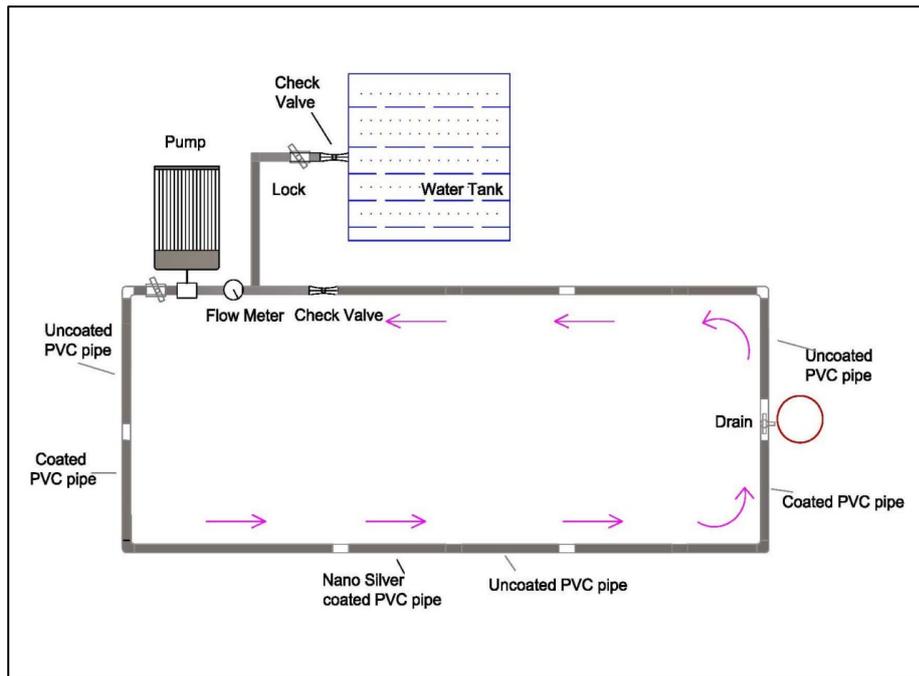


Fig. 3.6: Schematic diagram of the experimental setup.

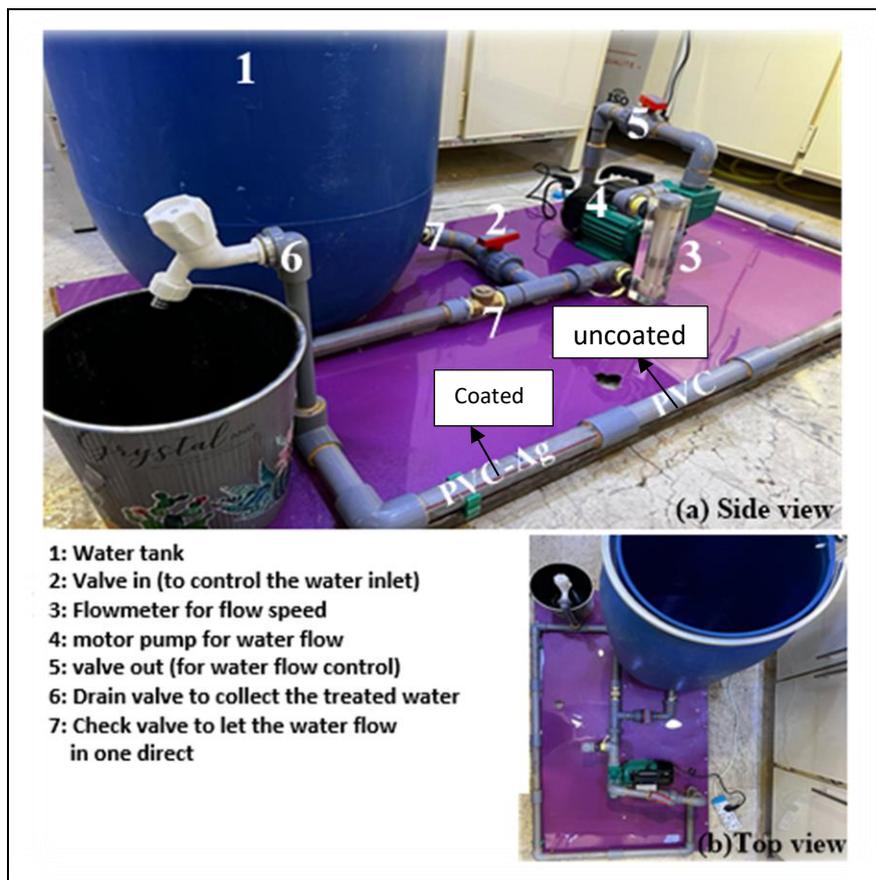


Fig. 3.7: Experimental setup, (a) Side view, (b) Top view.

3.7 Antibacterial Activity of AgNPs

3.7.1 In Vitro Antibacterial Activity Against of *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853

3.7.1.1 Standard Isolate of Bacteria

Standard isolates of *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were obtained from the laboratories of the Environmental Research Center / University of Technology / Baghdad / Iraq, to examine the effect of the AgNPs prepared by the green synthesis upon its viability in vitro where the two isolates have been grown and maintained on nutrient agar.

3.7.1.2 Preparation of Nutrient Agar

The culture media was prepared according to the manufacturer's (Gilmore et al., 2014) instructions as shown in Fig. 3.8 where 23 g of powder was dissolved in one liter of distilled water with stirring on a hot plate. Then, it was sterilized in an autoclave for 15 min at a temperature of 121°C and left to cool out to 50 °C and placed into sterile Petri dishes. Table 3.3, illustrates the ingredients of nutrient agar.



Fig. 3.8: Nutrient agar preparation.

Table 3.3: Ingredients of nutrient agar (Gilmore et al., 2014).

Ingredient	Concentration
"Lab-Lemco" Powder, g/L	1.00
Yeast extract, g/L	2.00
Peptone, g/L	5.00
Sodium chloride, g/L	5.00
Agar, g/L	15.00
pH	7.4 ± 0.2

3.7.2 Antibacterial Assay

The pour plate methods was carried out to testify the effect of AgNPs prepared at different concentrations (0.0, 125, and 250 ppm) on two isolates of bacteria (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853), through the steps shown below:

1. Bacteria cells were activated by culturing them in one liter of brain infusion broth (BIB) for 24 hours at 37 °C with continuous shaking.
2. The activated bacteria were collected after being centrifuged at 5000 rpm for 10 min and the pellet was washed with phosphate buffer saline three times.
3. The total number of activated cells was adjusted to (1×10^6) CFU/mL.
4. The activated cells were added to a water tank of 75-liter (free from chlorine), connected to a distribution system as described in paragraph 3.6, in which the water pipes were coated with silver nanoparticles at a known concentration each run (125 and 250 ppm), then the contaminated water was pumped into the distribution system at different times (2, 5, 10, 15 and 20 min).
5. After different exposure times, one milliliter of treated water was taken and mixed with a volume of 25 mL of Muller Hinton (MH) medium in a liquid state and was left for a quarter of an hour in the Biological

safety cabinet as shown in Fig. A.4. All plates were incubated for 24 hours at a temperature of 37 °C.

6. After the incubation period, the plates containing bacterial growth were taken to count the number of bacteria. The inhibition rate was calculated according to the following equation (Bauer et al., 2014).

$$\text{Inhibition ratio (\%)} = 100 - \left(\frac{\text{No. of colonies of test}}{\text{No. of colonies of control}} \times 100 \% \right) \dots\dots (3.1)$$

3.8 Physical, Chemical, and Biological Examination of Drinking Water

Several physical, chemical, and biological parameters of drinking water such as pH , EC, TDS, and total viable count (TVC) were measured after drinking water passed through the water pipes coated with two concentrations of AgNPs at different exposure times Iraqi Standard No. (.45) for the year 1984 .

3.9 Silver Content in the Effluent Water

Atomic Absorption Spectrometry (AAS) was used to assess silver content in effluent water sample after being passed through water pipes coated by 250 ppm silver nanoparticles according to the international methods (METHODS 3005A). 100 mL of water was taken at different times (2, 5, 10, 15, 20, 25 and 30 min), then 10 mL of nitric acid (HNO₃) was added and mixed thoroughly with the water sample, and the mixture was heated up to boiling temperature. After that left to cool out and filtered by Millipore filter paper of 0.45 µm. In the end, the volume was completed up to 100 mL by deionized water and tested by AAS as shown in Fig. (A.5).

3.10 Cytotoxicity of Silver Nanoparticles by MTT Assay

In vitro, cytotoxicity assay was applied by taking 10 mL of whole blood from 25 years old healthy man using venous puncture, the blood specimens was collected in glass tubes supported by anti-coagulant agent, where blood samples were centrifuged at 5000 rpm/min for 5 min. The floated plasma was ignored while the upper stratum (puffy WBCs) was gathered by glass Pasteur pipette and placed into a test tube containing 5 mL of sterile RPMI-1640 with 10% fetus veal serum having a definitive concentration of WBCs (1×10^4) cells/mL for the assessment of the venomous impact caused by the synthesized AgNPs on such blood cells. Consequently, the concentrations of AgNPs (125 and 250) ppm were tested by culturing the white blood cells in (RPMI-1640) media to assess the toxic effect. The examination was run out in a 96-well flat-bottom microtiter plate and all plates received 100 μ L of the WBCs suspension (1×10^4) cells/mL and subjected to tested material by adding them to the wells. The plates were incubated at 37 °C for 24 hours and the surplus amount of media and dead cells were eliminated.

The last row in at micro-titer plate (multi-wall) was considered as a negative control without any addition except the white blood cells and culture media, then rinsed with phosphate buffer saline (PBS) three times to remove all traces of AgNPs and unattached white blood corpuscles. 10 μ L of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used at an ultimate concentration of 0.5 mg/mL and added to each well and incubated approximately four hours at 37 °C.

Afterwards, these wells were rinsed several times with PBS to eliminate the surplus dye and the purple color was recorded by Formazan formation with the Elisa microplate spectrophotometer (Dana 3200, UK) as shown in Fig. (3.9) at a wavelength of 500 nm (McConkey et al., 1998; Balaji et al., 2016; Bezza et al., 2020).

The inhibition ratio was calculated according to equation (3.1) mentioned previously.



Fig. 3.9: Elisa Reader –Dana 3200, UK.

3.11 Hemocompatibility of AgNPs with Red Blood Cells (RBCs)

Hemolysis assay of AgNPs at different concentrations conducted according to previous studies by Balaji et al., (2016) and Al-Jubori et al., (2021). Human whole blood was collected by venous puncture from healthy persons in the age range (25-35) years old. The blood was collected in glass tubes supported by an anti-coagulant agent as Ethylene diamine tetra acetic acid (EDTA).

Thereafter, 0.5 mL of the whole blood was mixed with 4.5 mL of normal saline provided with different concentrations of AgNPs (125 and 250 ppm) in the direct exposure test. While 1 mL of water after passing via coated pipes at different exposures time was mixed with 3.5 mL of normal saline and 0.5 mL of the whole blood in the test of product water. Distilled deionized water was used at the positive control (100 % hemolysis), whereas normal saline was utilized as a negative control (0 % hemolysis).

All blood tubes were incubated at 37 °C for 1 hour and these tubes were centrifuged at 3000 rpm for 5 min.

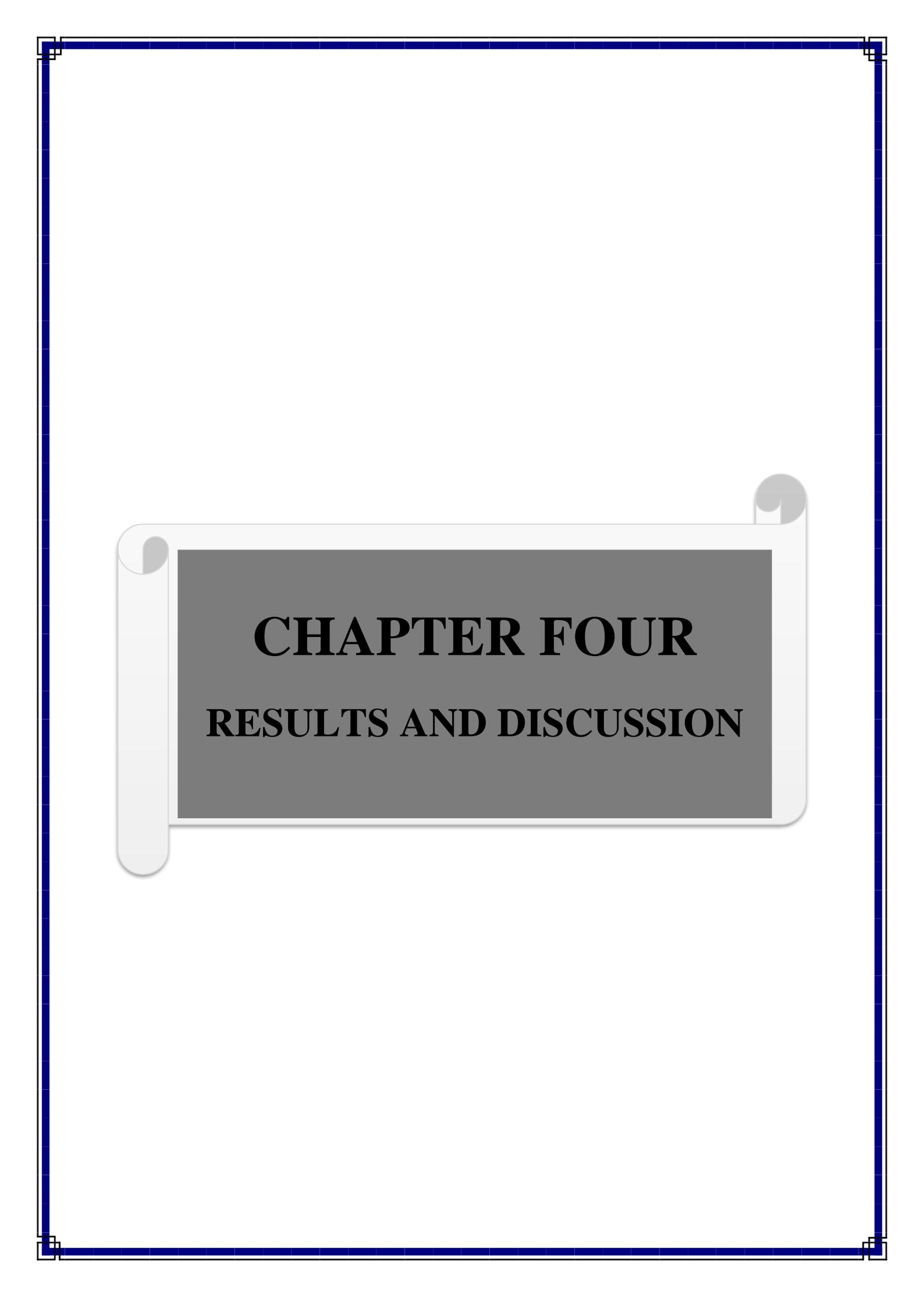
The percentage of hemolysis or hemolytic index was calculated according to the following formula:

$$\text{Hemolysis ratio (HR)} = \left[\frac{\text{Ab sample}}{\text{Ab (+ve)}} \times 100\% \right] \dots\dots\dots (3.2)$$

Where the absorbency (Ab) values of the test sample and positive control (+ve) were measured by UV-Vis spectrophotometer at 542 nm.

3.12 Statistical Analysis

The obtained data is statistically examined via analysis of variance (ANOVA) by applying SPSS software version 22. Also, the mean value \pm standard deviation (SD) were used for the experimental data (Mean \pm SD). In addition to test the least significant differences (LSD) were calculated at $P \leq 0.05$ for assessing the differences between examined variables.



CHAPTER FOUR
RESULTS AND DISCUSSION

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents and explains the obtained results about the characterization of silver nanoparticles using UV-Vis spectrophotometer, FTIR spectroscopy, FESEM, TEM, and XRD, as well as the characterization results of silver nanoparticles after the drinking water pipes coating process using FESEM and XRD.

It also discusses the results of the bacteriological analysis of water samples before and after passing through the AgNPs-coated drinking water pipes. Also, this chapter displays the measuring results of several physical and chemical properties of effluent water. The analysis of silver content in the effluent water was assessed by using AAS.

Finally, the cytotoxicity of AgNPs was evaluated by MTT assay and hemocompatibility of AgNPs with RBCs at various exposure times to different AgNPs concentrations.

4.2. Characterization of Synthesized Silver Nanoparticles

4.2.1. Ultraviolet–Visible (UV-Vis) Spectrum

Colloidal solutions containing silver nanoparticles were prepared using the green synthesis methods and have given a unique peak at 422 nm as shown in Fig. (4.1), which is attributed to the Surface Plasmon Resonance (SPR). This value fits with the SPR peaks recorded previously and therefore, this measurement proved the success of the formation of silver nanoparticles. The same finding was reported by previous studies (Jasem et al., 2019; Sikes et al., 2022).

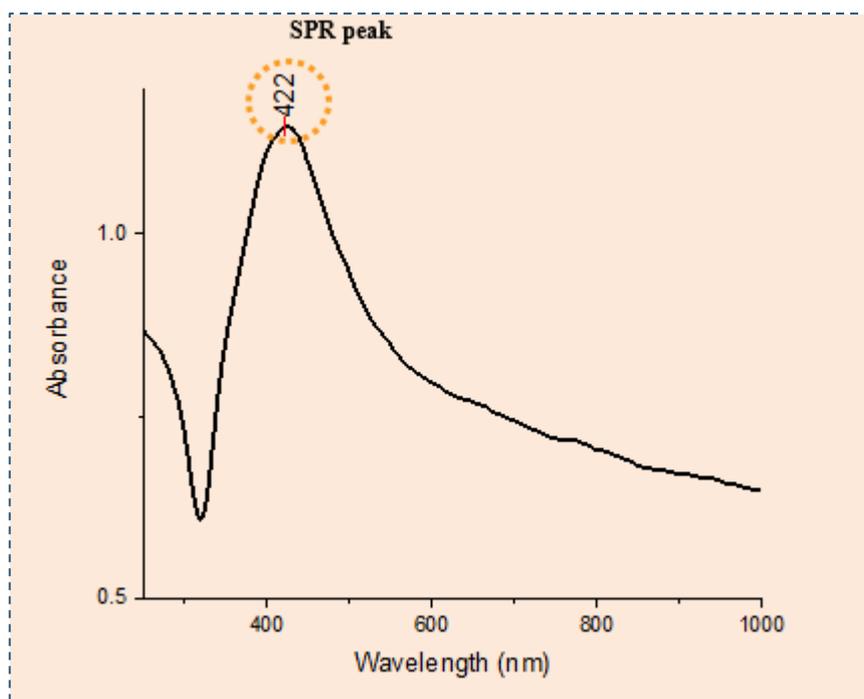


Fig. 4.1: UV-Vis spectrum of the green synthesized silver nanoparticles.

4.2.2. Fourier Transform Infrared (FTIR) spectrum

This measurement is obviously used to determine the active groups present in the chemical compound, but it is usually applied to diagnose bonds that can vibrate with infrared (IR) energy, which are the bonds in organic and coordinating materials. Based on this, this measurement is not used in the diagnosis of metallic bonds, so it is usually not utilized for metallic materials, including silver nanoparticles. However, this measurement is of importance in determining the effective aggregates of the materials present on the surface of the prepared silver nanoparticles as shown in Fig. 4.2, where the measurement showed the presence of many functional groups, which indicates the presence of active substances on their surface.

The measurement showed the presence of a high-intensity wide band at 3421 cm^{-1} in addition to another band at 1639 cm^{-1} that counted for the expansion and curvature oscillations of the O-H bond present in water, which is present due to the adsorption of water on the surfaces of silver nanoparticles because it has a very high surface area.

The measurement also showed the presence of the following bands at 1161, 1411, (1546/1608), 1701, and (2837/2958), these findings were due to the expanded vibration of C-O-C, C-O, (C=C), C=O and -C-H groups, respectively. The presence of these functional groups confirmed the presence of phenols, alkaloids, carboxylic acids, as well as aromatic compounds and aliphatic substitutes or aliphatic chains. This result was compatible with the result obtained by Barbhuiya et al., (2022) and Shahabadi et al., (2022).

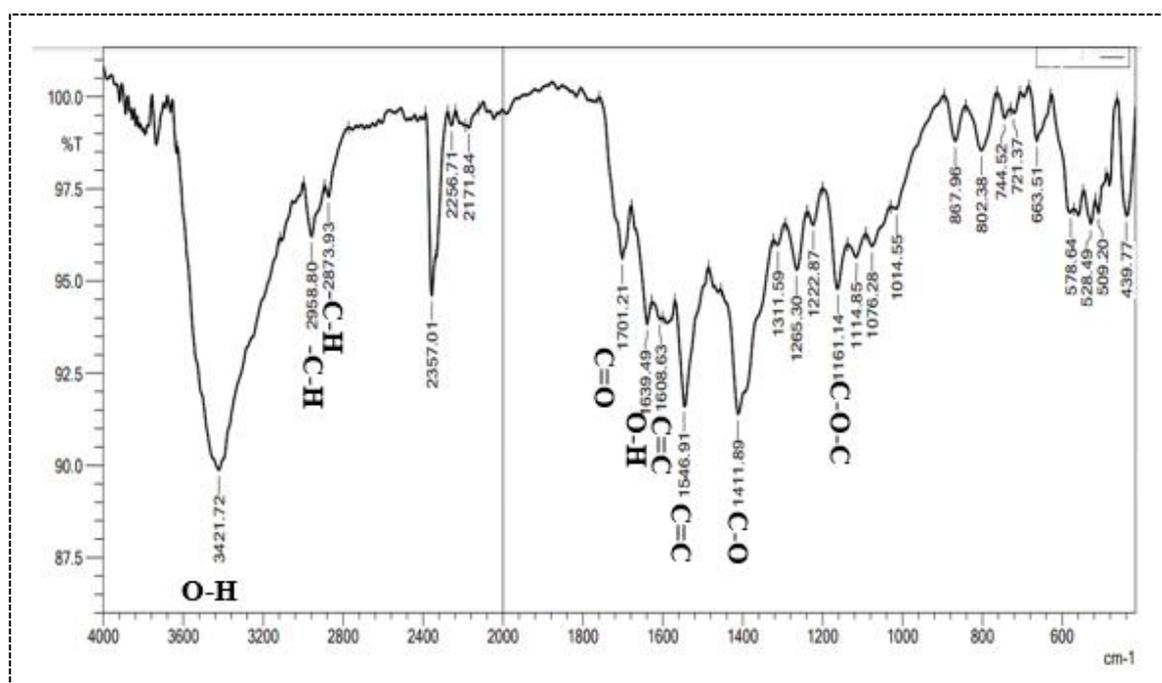


Fig. 4.2: FTIR spectrum of the green synthesized silver nanoparticles.

4.2.3. X-Ray Diffraction (XRD)

This measurement aims to determine the type of substance or compound as well as its purity. This technique has been widely used in the characterization of nanomaterials and the determination of particle size using the Debye-Scherrer equation. The XRD measurement was displayed in Fig. 4.3 which presents four main peaks at 38.190032°, 44.440125°, 64.740340° and 77.741242° that are the characteristic peaks of pure Nano- silver with Full Width High Maximum (FWHM) values larger than 0.2458°. This result was

agreed with Abdulkareem et al., (2022) and Dhanam et al., (2021). These peaks belong to Face-Centered Cubic (FCC) silver because they correspond to crystal planes 111, 200, 220, and 311, respectively. Moreover, the FWHM indicated the presence of silver in the nanoscale, which is further proved by the Debye Scherer equation (Wang, 2000) as given below:-

$$\tau = (K*\lambda)/ (\beta*\text{Cos} (\theta)) \quad \dots\dots\dots 4.1$$

Where τ : nanoparticle size, nm

K: is a unit less constant that depends on the shape of the crystal, often in the order of = 0.9

β : Width of the peak at average height, (radian $\times 10^{-3}$)

$\beta = \text{FWHM}/180$

θ : Prague corner, ($^{\circ}$)

Where the particle size for each peak was calculated, which averaged 31.81 nm, as shown in Table 4.1.

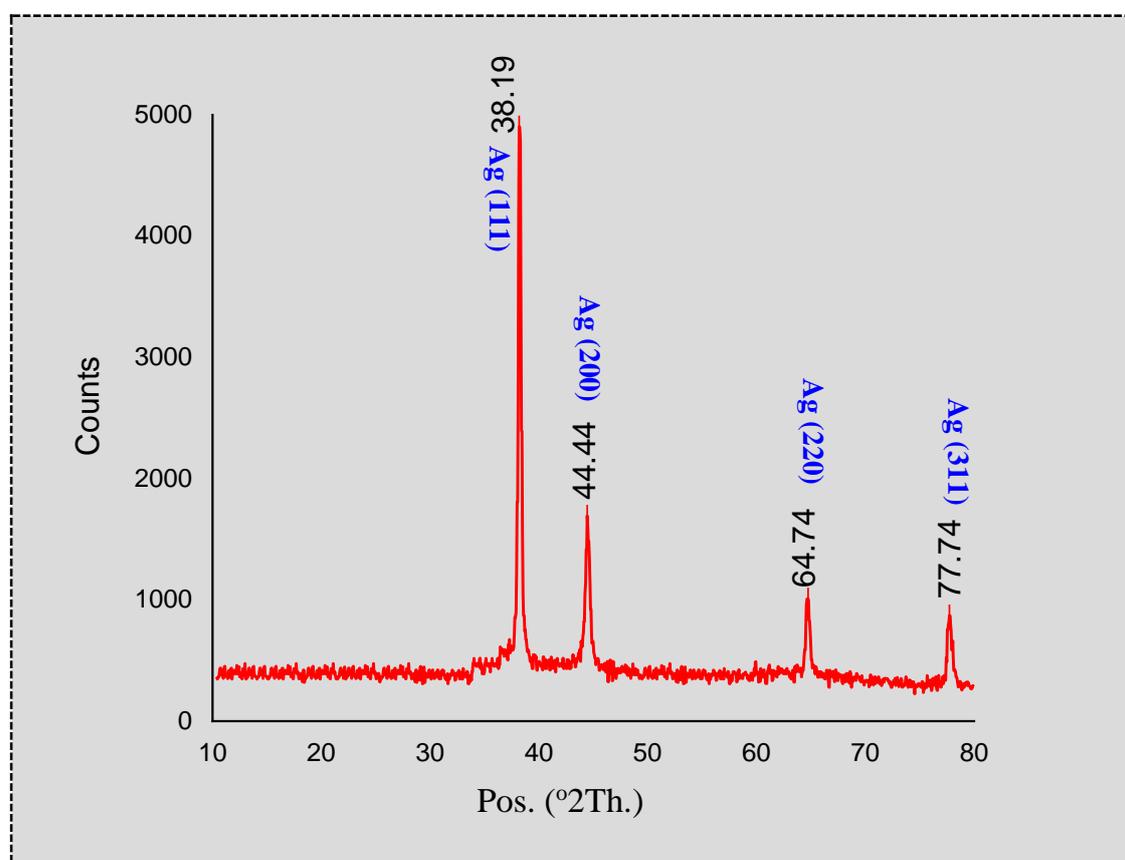


Fig. 4.3: XRD measurement of the green synthesized silver nanoparticles.

Table 4.1: XRD information and average particle size.

Pos., ($^{\circ}$ 2Th.)	Height, (CTS)	FWHM Left, ($^{\circ}$ 2Th.)	D, (nm)	D average, (nm)	Index
38.190032	4374.01	0.294910	29.8000	31,8100	111
44.440125	1214.94	0.294910	30.4200		2 0 0
64.740340	670.14	0.245800	40.0000		2 2 0
77.741242	539.92	0.394300	27.0500		3 1 1

4.2.4. Field Emission Scanning Electron Microscopy (FE-SEM)

After the preparation of AgNPs, an investigation of the presence of AgNPs, there are usually characterized by an imaging technique (FE-SEM) in order to determine the shape of nanoparticles. Silver nanoparticles are usually prepared in a spherical shape; therefore this shape is considered as the most popular one. In terms of application in removing biological pollutants, this shape has an effective role in being the form with the largest surface area.

In this study, silver nanoparticles were characterized using FE-SEM. The results are presented in Fig. (4.4), which proved the presence of silver nanoparticles in a spherical shape and sizes ranging between 40-90 nm which indicates the success of the preparation methods. This, however was proven by the particle size distribution chart (Fig. 4.5). When comparing the particle size calculated depending on the Scherrer equation from the XRD measurement with the size results in FE-SEM, it was found that the values are somewhat close and the reason for the difference is mainly because the fact that the equation is not accurate, therefore the size determined from the FE-SEM technique is the most comprehensive.

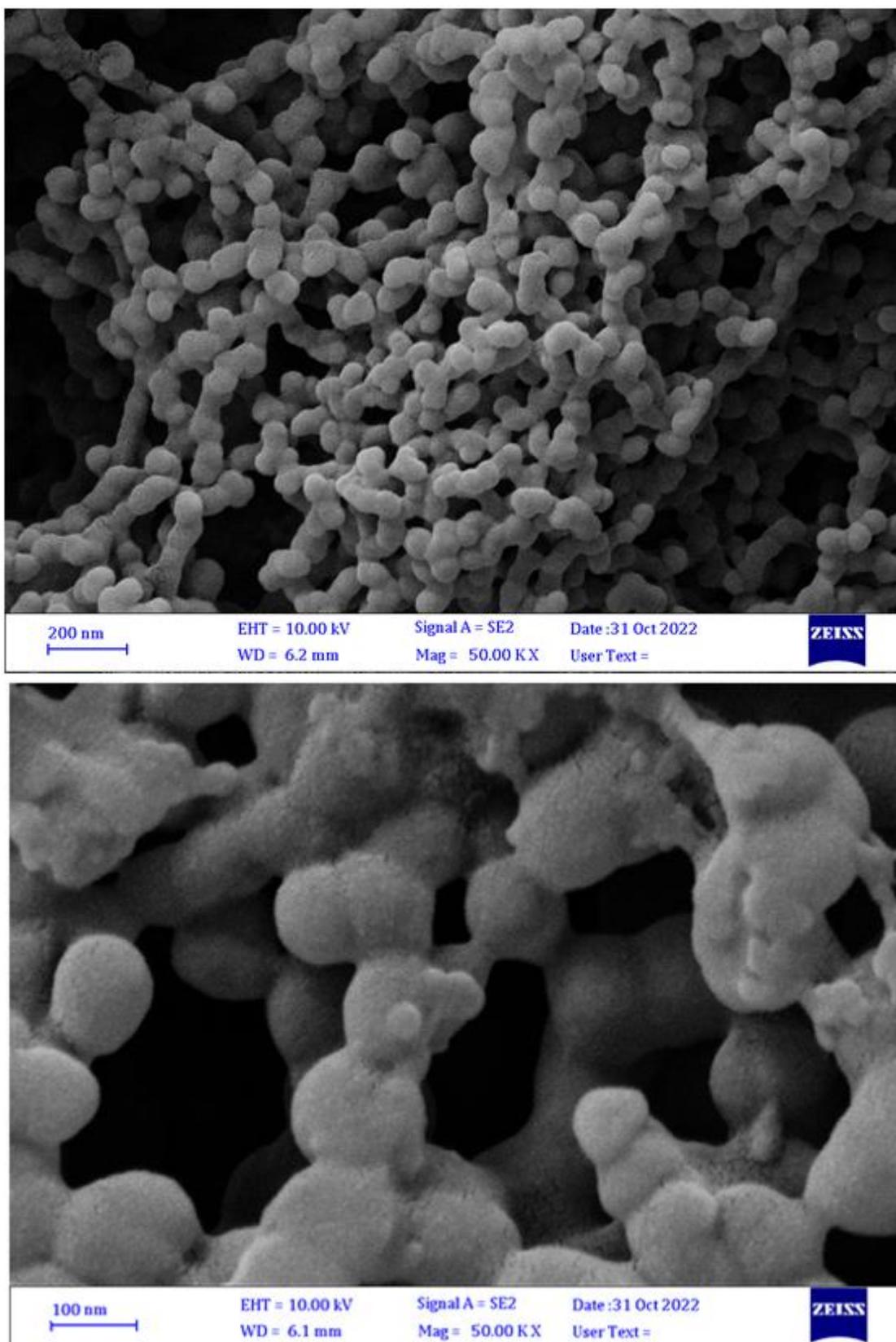


Fig. 4.4: FE-SEM measurement of the green synthesized AgNPs.

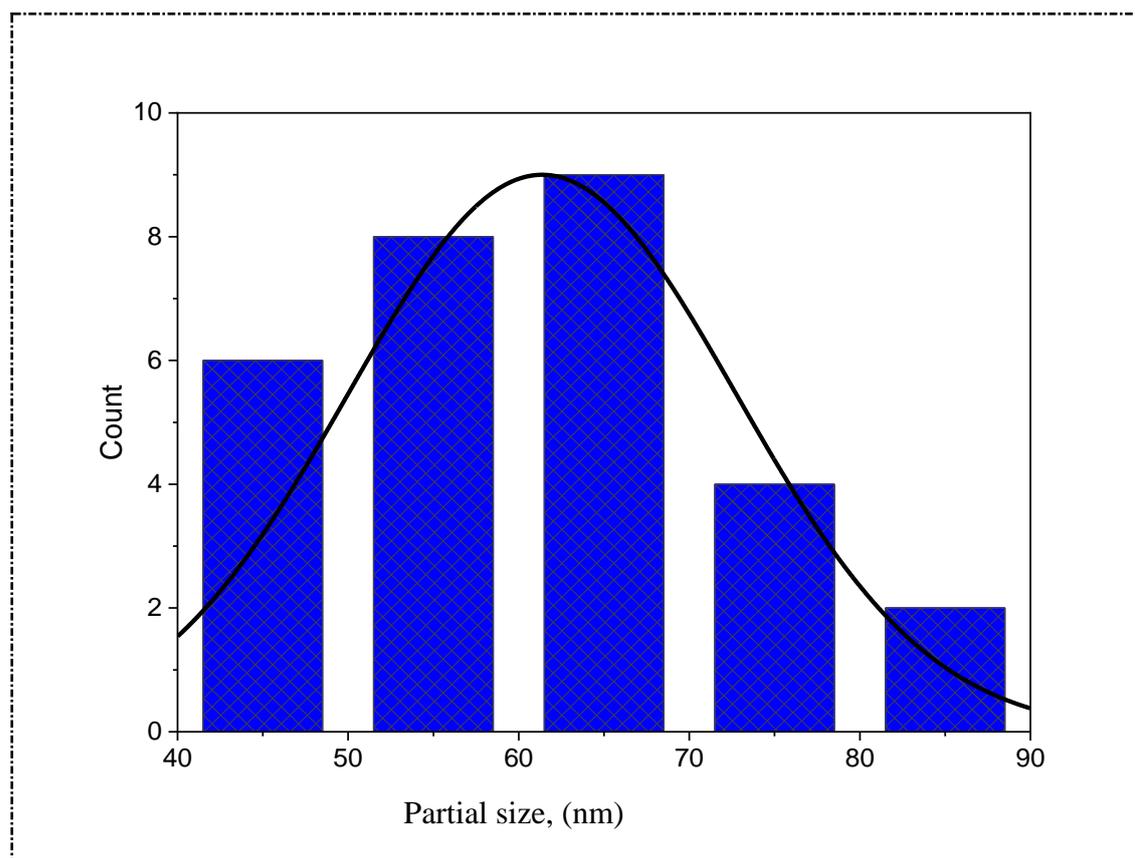


Fig. 4.5: Particle size distribution of the green synthesized AgNPs

4.2.5 Transmission Electron Microscopy (TEM) measurement

In order to obtain the exact shape and size of the prepared silver nanoparticles, in addition to realizing the extent of the separation of the particles from each other, this measurement was required.

Fig. (4.6) and Fig. (4.7) present the TEM measurement of silver nanoparticles prepared using the bioreduction methods (biosynthesis). The measurement showed the presence of particles with irregular spherical shape and sizes ranging between 35 and 70 nm. This indicates the success of the used preparation methods, thus the possibility of using it in the existing application procedure. Furthermore, the measurement explains that there is a high separation of the particles from each other. Therefore, the possibility of their aggregation could be non-existent, and therefore obtaining efficient performance in the application would be inevitable.

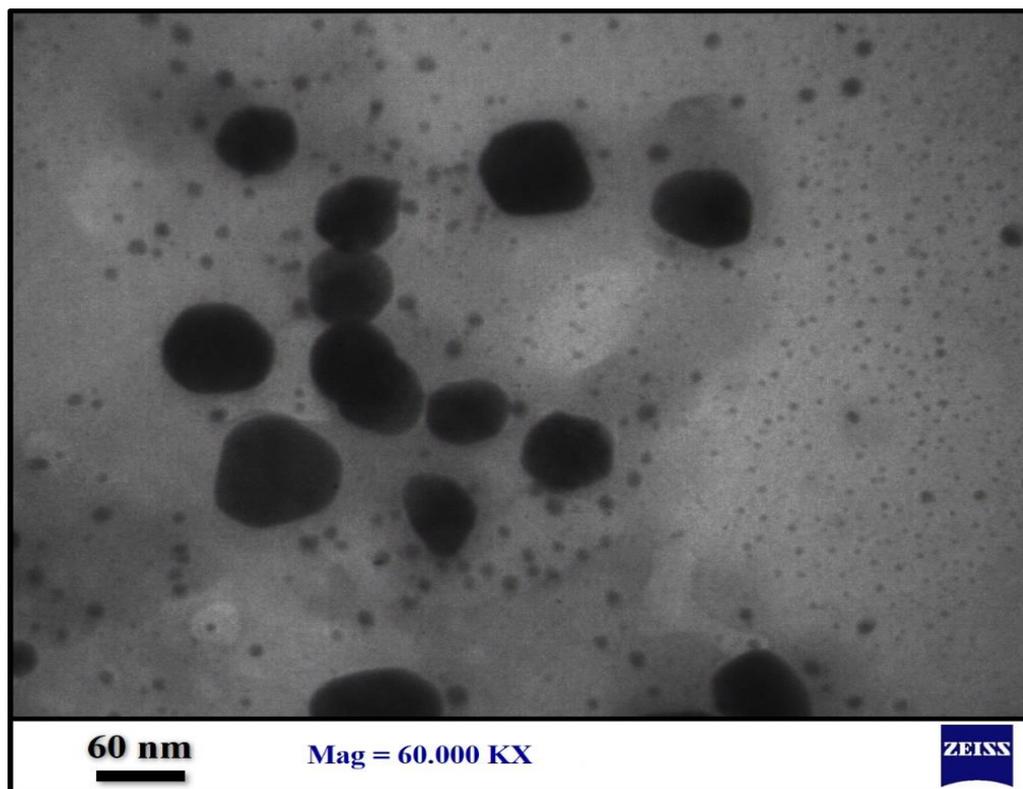


Fig. 4. 6: TEM measurement of AgNPs prepared by using the green synthesis methods.

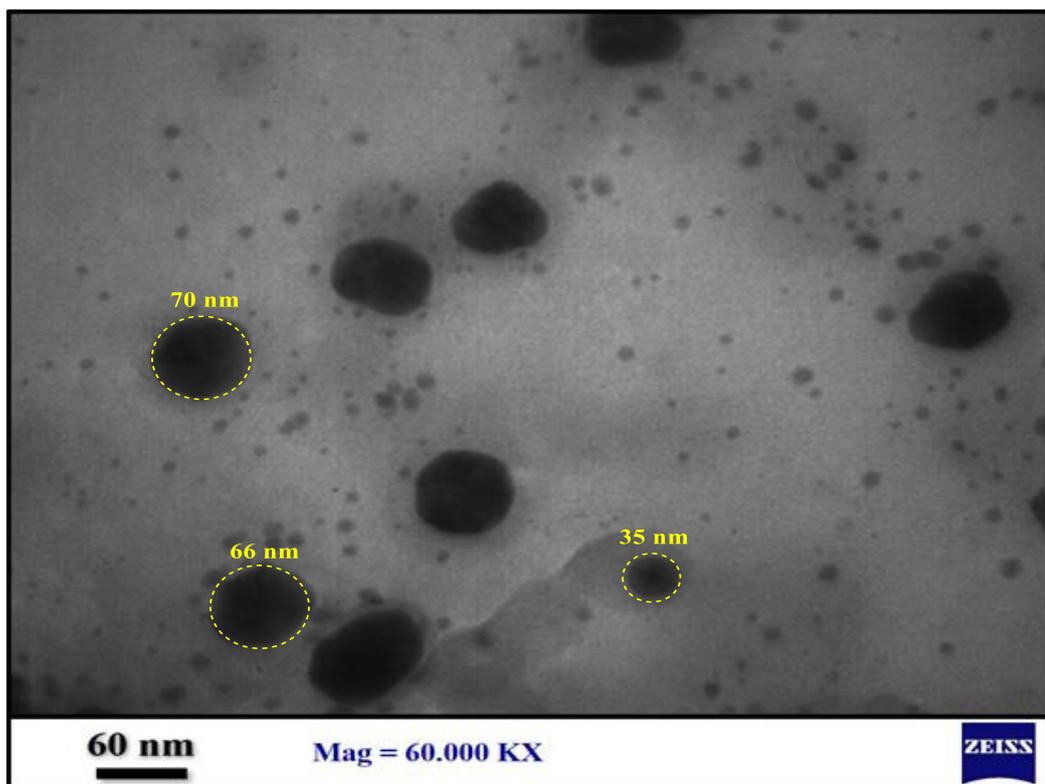


Fig. 4.7: Particle size of AgNPs measured by TEM.

4.3. Characterization of PVC pipes coated with AgNPs

4.3.1. FE-SEM

The PVC drinking water pipes were coated with silver nanoparticles using the hot air current annealing methods. Concentrations of silver (125 and 250) ppm were followed to determine the sufficient concentration and its relationship with the time factor. These pipes were characterized using FE-SEM and XRD techniques in order to determine the differences resulting from the change in concentration. The results are shown in Fig. (4.8) and Fig. (4.9). The measurement has proven that the coating methods was successful because the surface contained sphere-like particles with sizes ranging from 22 to 29 nm in case of using the concentration of 125 ppm, while sizes ranged from 24 to 32 nm for the coated pipes with a concentration of 250 ppm.

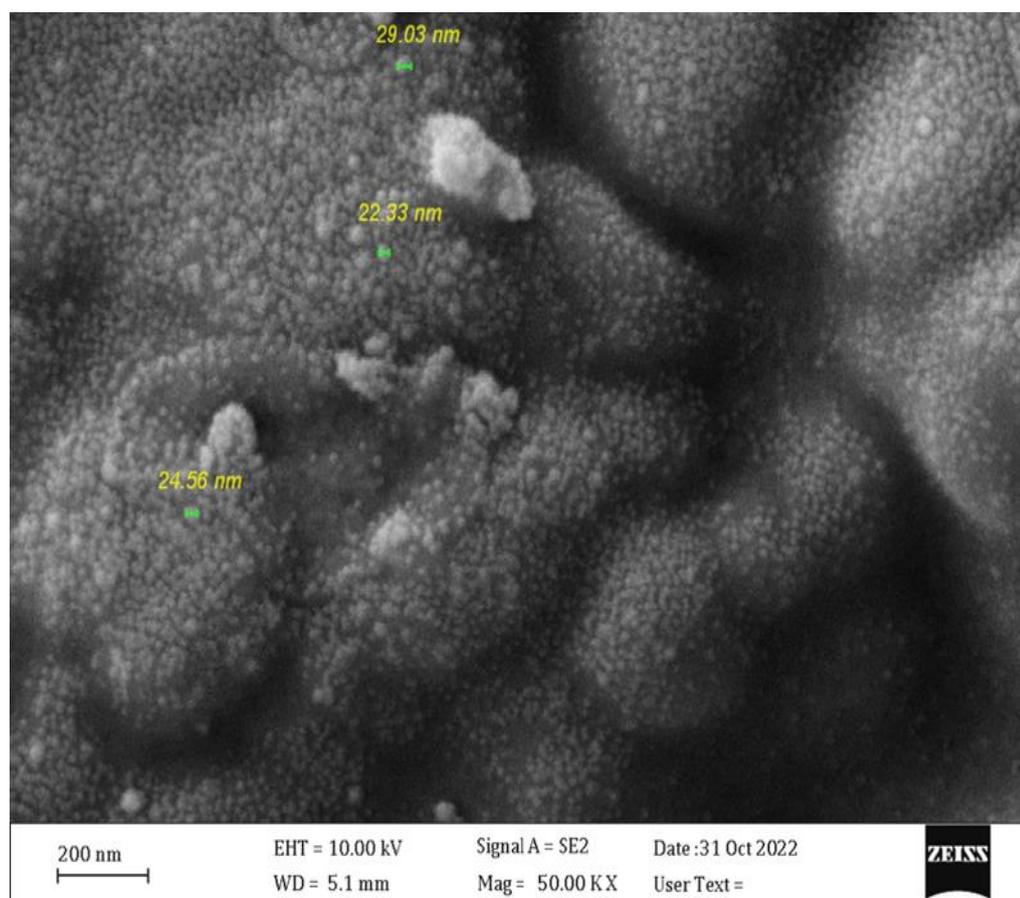


Fig. 4.8: Inner surface of the PVC pipe coated with silver nanoparticles (125 ppm).

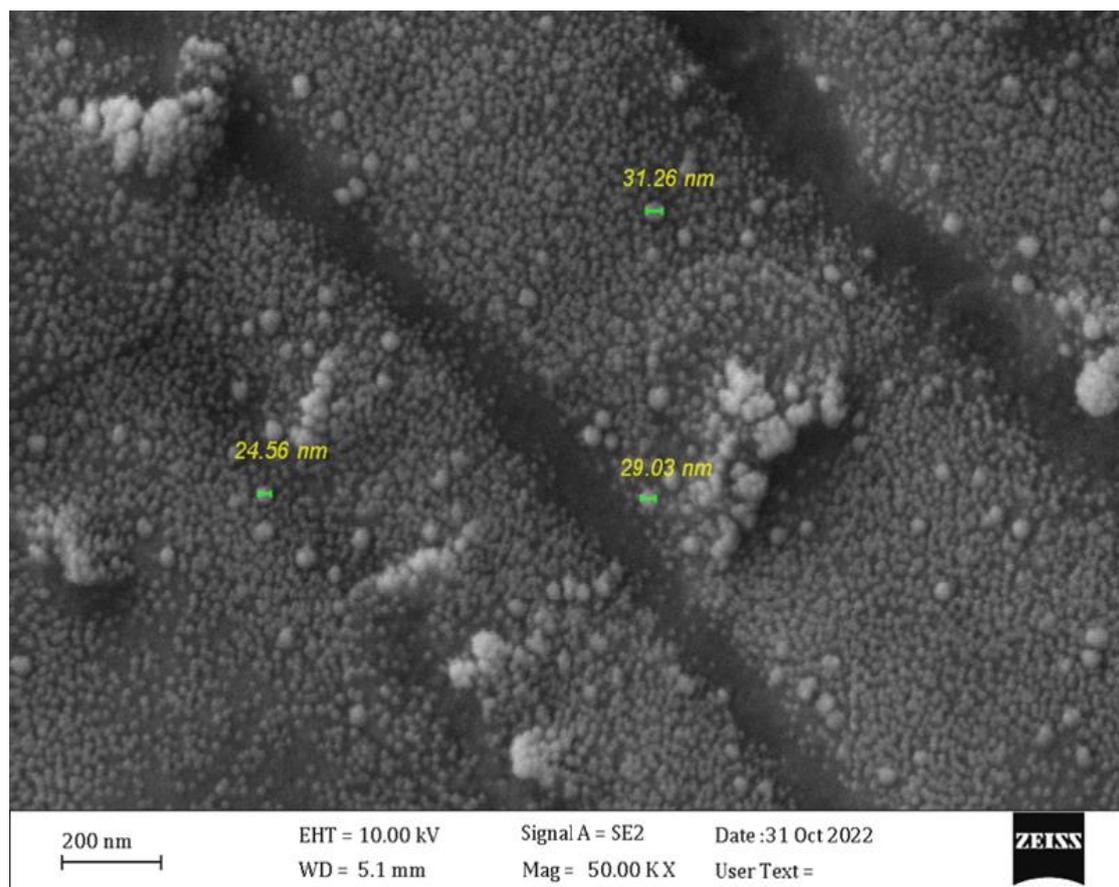


Fig. 4.9: Inner surface of the PVC pipe coated with silver nanoparticles (250 ppm).

As expected, the use of a lower concentration has given a relatively smaller particle size, and the measurement showed that the silver coating was more at a concentration of 250 ppm, which confirms that there is a great inhibition of bacterial growth and killing.

4.3.2. XRD

This technique was applied to determine the presence of silver on the inner surface of the water pipe. In addition, this technique confirmed that no changes occurred in the silver nanoparticles after heat treatment using hot air on the surface of the pipe. The examination proved the presence of Nano-silver because it showed its distinctive peaks at 39.593° , 47.497° , 57.507° and 64.943° , which were observed to have shifted to different positions than the silver nanoparticles alone, indicating that the silver adhered in a reaction manner on the inner surface of the pipes. Moreover, the measurement was

presented in Fig. (4.10) and showed the presence of other peaks where the red line is for PVC-AgNPs (250 ppm), and the black line is for PVC-AgNPs (125 ppm), which are attributed to PVC. These results were consistent with those of a previous study by Taha, (2019). It was remarkable that the small size of the nanoparticles has been observed after their interaction on the surface of the pipe, where the size of the particles was calculated based on the characteristic peaks of silver only and reached 15.255 nm (Table 4.2), which corresponded to an extent significant with the results recorded in the FE-SEM measurement.

The examinations have found that both water pipes have a perfect match in the XRD measurement except for the intensity of the silver peaks due to the addition of two different concentrations, so the higher concentrations gave a higher intensity.

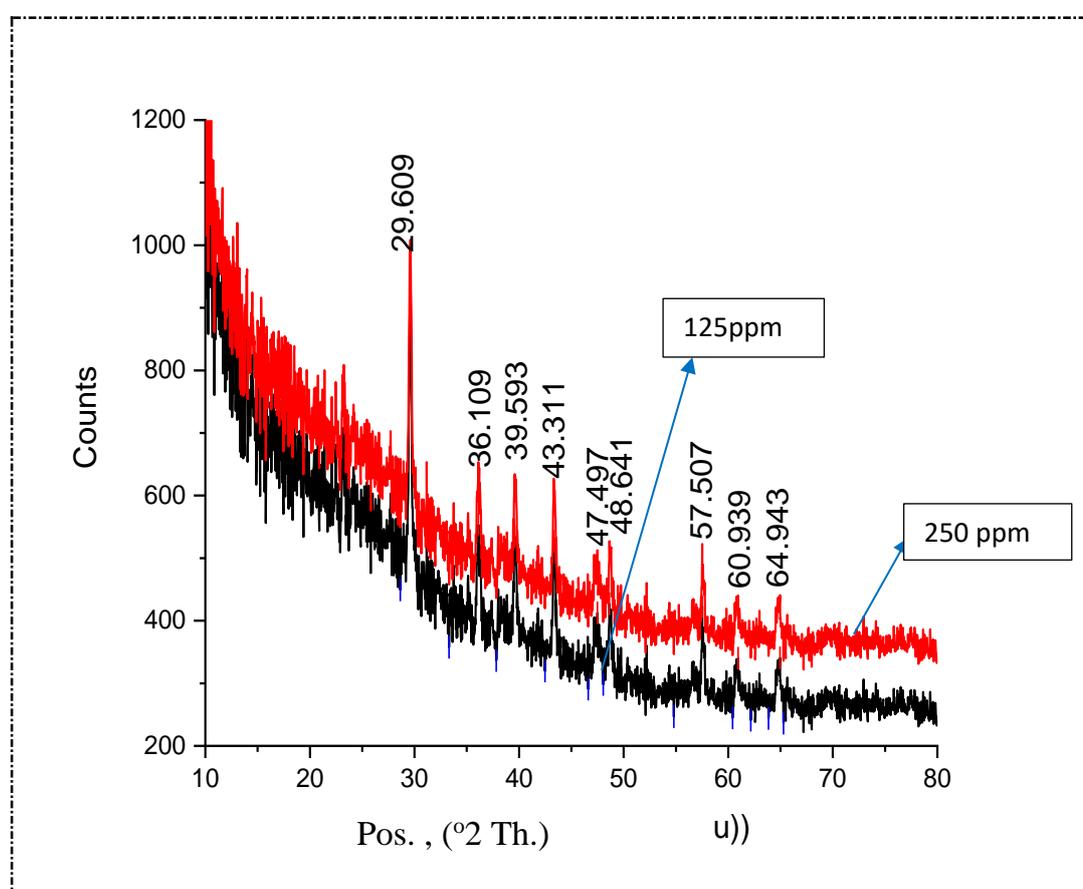


Fig. 4.10: XRD measurement of PVC pipes coated with AgNPs.

Table 4.2: XRD information and average silver particle size for PVC pipes coated with AgNPs.

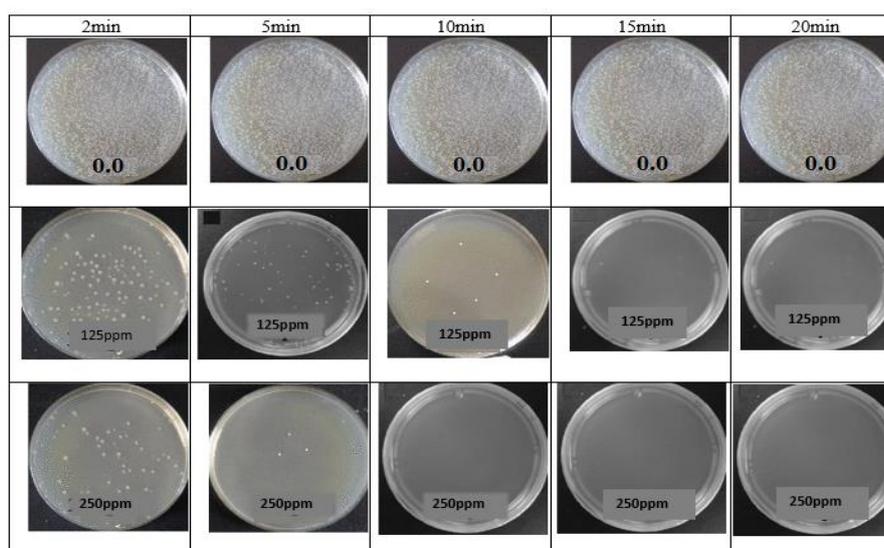
Pos. , (°2Th.)	FWHM Left, (°2Th.)	D, (nm)	D average, (nm)	Assignments
29.609	0.49023	-	15.2550	PVC
36.109	0.71686	-		PVC
39.593	0.69729	12.6600		Ag NPs
43.311	0.50815	-		PVC
47.497	1.03428	08.7700		Ag NPs
48.641	0.65772	-		PVC
57.507	0.4023	23.5400		Ag NPs
60.939	0.55432	-		PVC
64.943	0.6131	16.0500		Ag NPs

4.4 Antibacterial Activity of AgNPs

From the results given in Table 4.3, it was noticeable when exposing *E.coli* bacteria to different concentrations of silver nanoparticles by passing water containing bacteria (280 CFU/mL) , in a water pipe coated with AgNPs, there was a noticeable decrease in the colonies number on the surface of solids agar (Muller Hinton) depending mainly on the concentration of silver nanoparticles and the exposure time as shown in Fig. (4.11) and Fig. (4.12) where the inhibition percentage increased significantly at the probability level ($P \leq 0.05$) in case of increasing both the concentration and exposure time.

Table 4.3: Colony count of *Escherichia coli* ATCC 25922 exposed to different concentrations of AgNPs and different exposure time.

Concentration of coating (ppm)	<i>Escherichia coli</i> ATCC 25922, CFU/mL					
	Time (min), pipe 30 cm in length					
	0.0	2	5	10	15	20
0.0	280±25	280±15	280±13	280±11	280±10	280±15
125	280±13	80±7*	50±1*	5±10*	0.0*	0.0*
250	280±25	50±1*	3±0.0*	0.0*	0.0*	0.0*
<ul style="list-style-type: none"> * significant at $p < 0.05$ P value = 0.27023 Each number represent (M±SD) for three replicates 						
Concentration of coating (ppm)	<i>Escherichia coli</i> ATCC 25922, CFU/mL					
	Time (min), pipe 50 cm in length					
	0.0	2	5	10	15	20
0.0	280±25	280±22	280±21	280±20	280±18	280±12
125	280±12	120±11*	60±3*	32±1*	2.0±0.0*	0.0*
250	280±20	32±2*	20±2*	1.0±0.0*	0.0*	0.0*
<ul style="list-style-type: none"> * significant at $p < 0.05$ P value = 0.326379 Each number represents (M±SD) for three replicates 						

**Fig. 4.11:** Colony count of *Escherichia coli* ATCC 25922 exposed to different concentrations of AgNPs and different exposure time, (30cm) pipes length.

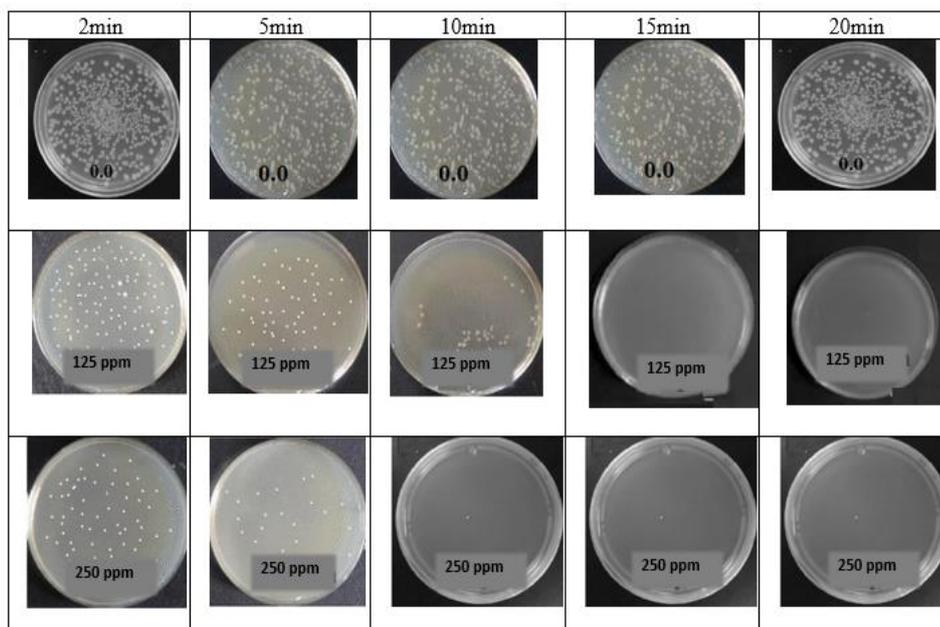


Fig. 4.12: Colony count of *Escherichia coli* ATCC 25922 exposed to different concentrations of AgNPs and different exposure time, (50cm) pipes length.

It was found in the case of using water pipes with a length of 30 cm that the inhibition rate reached 100% at 125 ppm after 15 min, but in the case of a concentration of 250 ppm, the inhibition rate has achieved 100% after 10 min as shown in Fig. 4.13. In case of a 50 cm water pipe length, the inhibition rate reached 100% after 20 min at concentration of 125 ppm, while at 250 AgNPs ppm, the inhibition rate attained 100% after 15 min as shown in Fig. (4.14).

From the above results, it seems that the rate of inhibition depends mainly on exposure time and silver nanoparticles concentration.

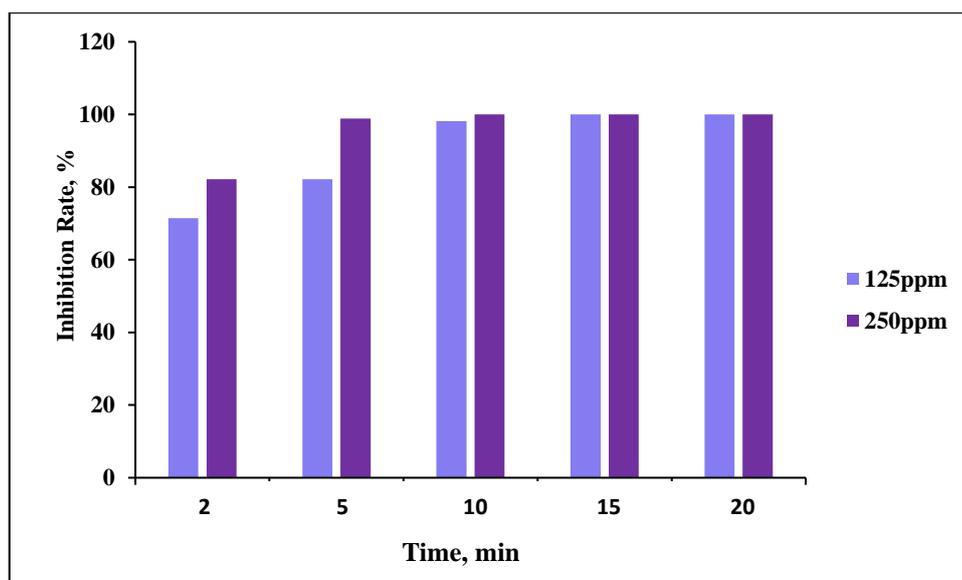


Fig. 4.13. Inhibition rate of *Escherichia coli* ATCC 25922 at different concentration of AgNPs with different exposure time, (30cm) pipes length.

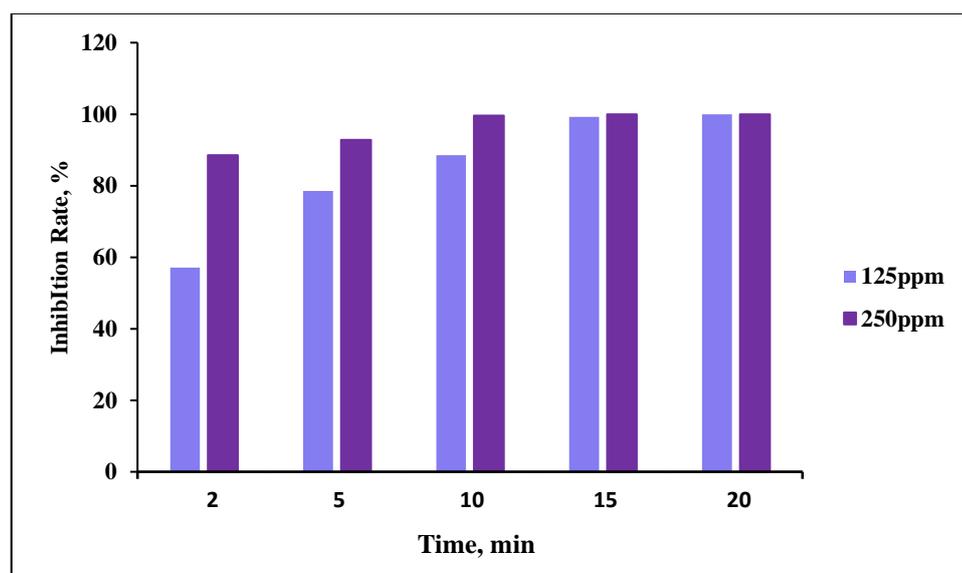


Fig. 4.14: Inhibition rate of *Escherichia coli* ATCC 25922 at different concentrations of AgNPs with different exposure time, (50cm) pipes length.

In the case of exposing *P. aeruginosa* bacteria to 125 and 250 ppm concentrations of silver nanoparticles via passing water containing bacteria in a water pipe coated with AgNPs as given in Table 4.4, there was a clear decrease in the number of colonies developed on a medium (Muller Hinton) as shown in Figures 4.15 and 4.16. The inhibition percentage has increased significantly at

the probability level of $P \leq 0.05$ in the case of increasing both AgNPs concentration and the exposure time.

Table 4.4: Colony count of *P. aeruginosa* ATCC 27853 exposed to different concentrations of Ag NPs and different exposure times.

Concentration of coating (ppm)	<i>pseudomonas aeruginosa</i> ATCC 27853, CFU/mL					
	Time (min), (30cm) length					
	0.0	2	5	10	15	20
0.0	290±25	290±25	290±25	290±25	290±25	290±25
125	290±25	200±18*	102±12*	31±2*	5±0.6*	1±0.02*
250	290±25	150±13*	23±0.66*	0.0*	0.0*	0.0*
<ul style="list-style-type: none"> * significant at $p < 0.05$ P value = 0.273742 Each number represent (M±SD) for three replicates 						
Concentration of coating (ppm)	<i>pseudomonas aeruginosa</i> ATCC 27853, CFU/mL					
	Time (min), (50cm) length					
	0.0	2	5	10	15	20
0.0	290±25	290±25	290±25	290±25	290±25	290±25
125	290±25	209±28*	140±11*	66±3*	12±0.5*	4.0±0.5*
250	290±25	180±12*	54±2*	2.0±0.02*	0.0*	0.0*
<ul style="list-style-type: none"> * significant at $p < 0.05$ P value = 0.269858 Each number represent (M±SD) for three replicates 						

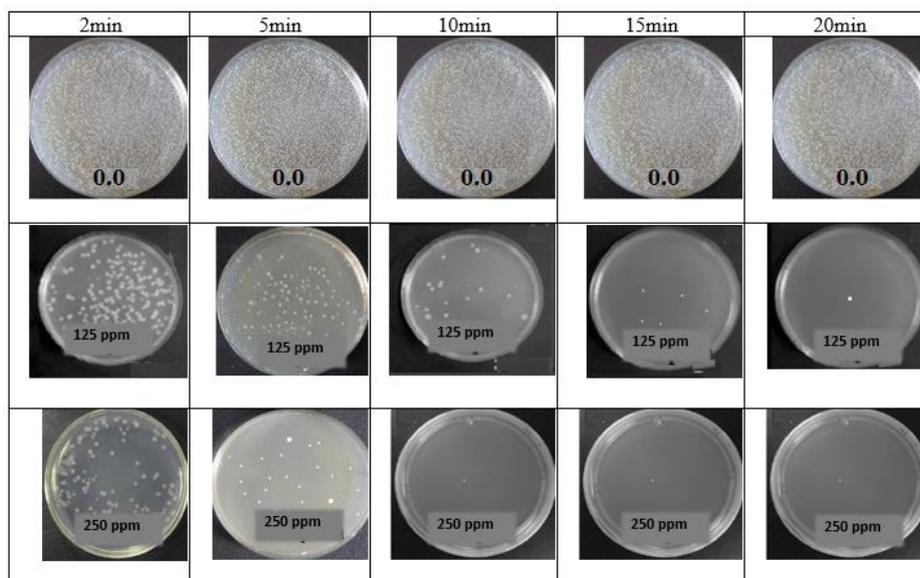


Fig. 4.15: Colony count of *P. aeruginosa* ATCC 27853 exposed to different concentrations of AgNPs and different exposure times, (30cm) pipes length.

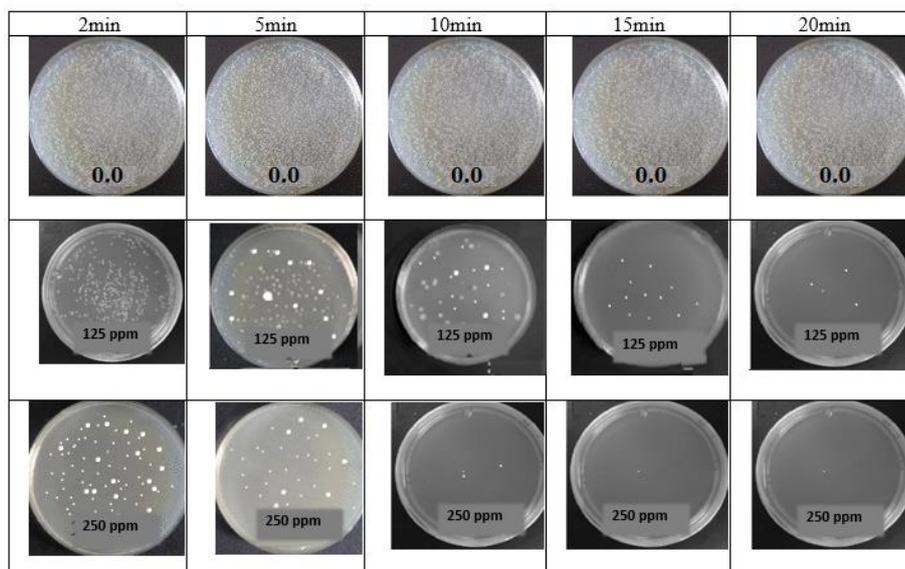


Fig. 4.16: Colony count of *P. aeruginosa* ATCC 27853 exposed to different concentrations of AgNPs and different exposure times, (50cm) pipes length.

It was found in the case of using a 30 cm pipes length, the inhibition rate attained 99.65% at concentration of 125 ppm after 20 min, but at a concentration of 250 ppm, the inhibition rate achieved 100% after 10 min as shown in Fig. (4.17) when using a 50 cm pipes length, the inhibition rate has arrived at

98.69% of 125 ppm after 20 min, but at 250 ppm, the inhibition rate has recorded 100% after 15 min as shown in Fig. (4.18).

From the results, it seemed clear that the rate of killing bacteria depends mainly on the exposure time and the concentration of silver nanoparticles.

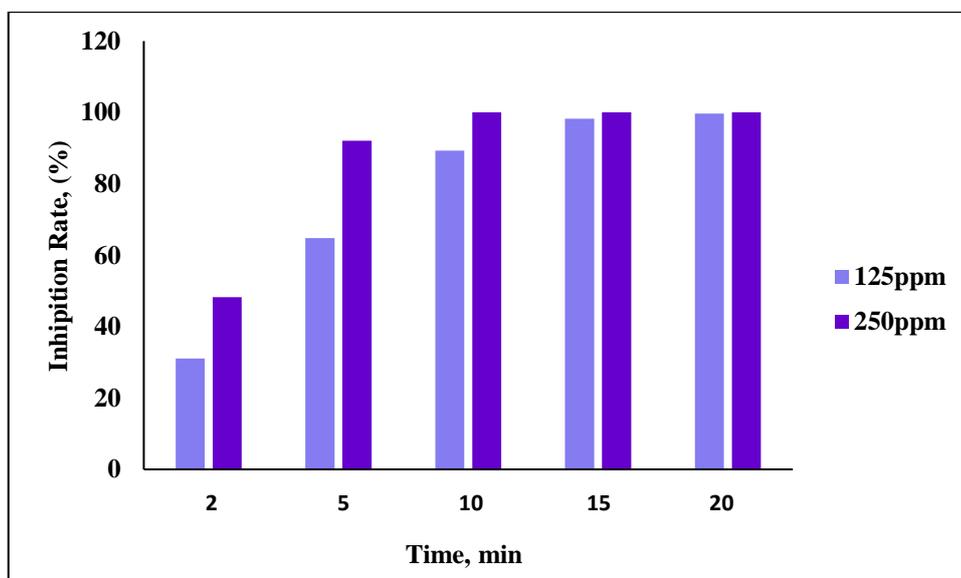


Fig. 4.17: Inhibition rate of *Pseudomonas aeruginosa* ATCC 27853 at different concentrations of Ag NPs with different exposure times, (30cm) pipes length.

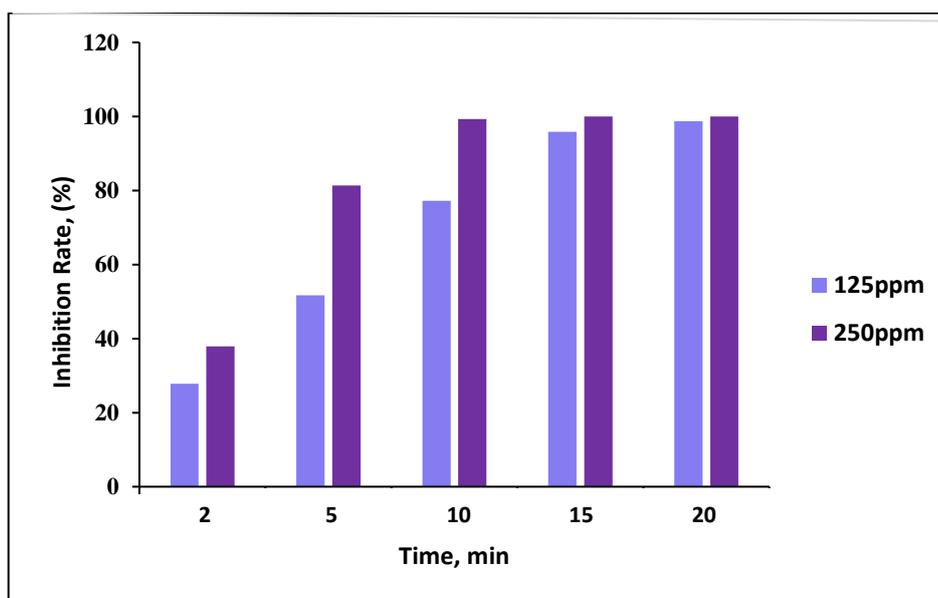


Fig. 4.18: Inhibition rate of *Pseudomonas aeruginosa* ATCC 27853 at different concentrations of Ag NPs with different exposure times, (50cm) pipes length.

The AgNPs were good-documented as an antimicrobial agent because of their unique properties, against many species of bacteria *E. coli* and *P. aeruginosa*.

An aqueous media can configure the biofilm, which adhered to microbial communities, enveloped by self-produced polymeric extracellular materials. Most microorganisms can attach to the inner pipe's surfaces and develop biofilm, which became the main source of biological water contamination. Polymeric materials like PVC were preferred in water distribution systems because they are simpler to use than other iron-based materials, in the current study, the growth rate and viability of both *E. coli* and *P. aeruginosa* in the drinking water passed through PVC pipes coated with different concentrations of AgNPs at different exposure time. It was important to examine the impact of AgNPs on the activity and survival of such types of bacteria.

From the mentioned above, despite reaching the permissible bacteria presence percentage, the concentration of silver nanoparticles within the length of the water PVC pipe had a clear effect on the time required to end the bacterial presence, because the fact that the surface area is greater in the case of a higher concentration of silver. Also, it causes more bacteria to be hindered by the pores on the surface and then allow time to interact with the heterogeneous atoms such as sulfur and nitrogen in the amino acids after their influence from the cell wall of the bacteria which agreed with the results obtained by Li et al., (2010) and Panáček et al., (2018). The higher concentration, the better in terms of obtaining a positive result within a short period, which has a good effect from an applied point of view.

4.5 Water Quality Examinations

Certain water quality variables such as pH, electrical conductivity, and dissolved solids were examined to evaluate the effects of AgNPs used in coating PVC pipes for drinking water before passing through the coated pipes.

The pH value was about 7.6, while the EC value was 1390 $\mu\text{s}/\text{cm}$, and finally the TDS value was about 833 mg/L. Fig. 4.19 to 4.21 represent the effects of AgNPs on these variables after passing water through AgNPs-coated water pipes. These variables were examined at two concentrations (125 and 250 ppm) of AgNPs that coated both 30 and 50 cm the length of PVC water pipes.

It was found that the pH values varied from 7.5 in 30 cm coated pipes at 10 and 20 min to 8.1 in 50 cm coated pipe after 25 min (Fig. 4.19).

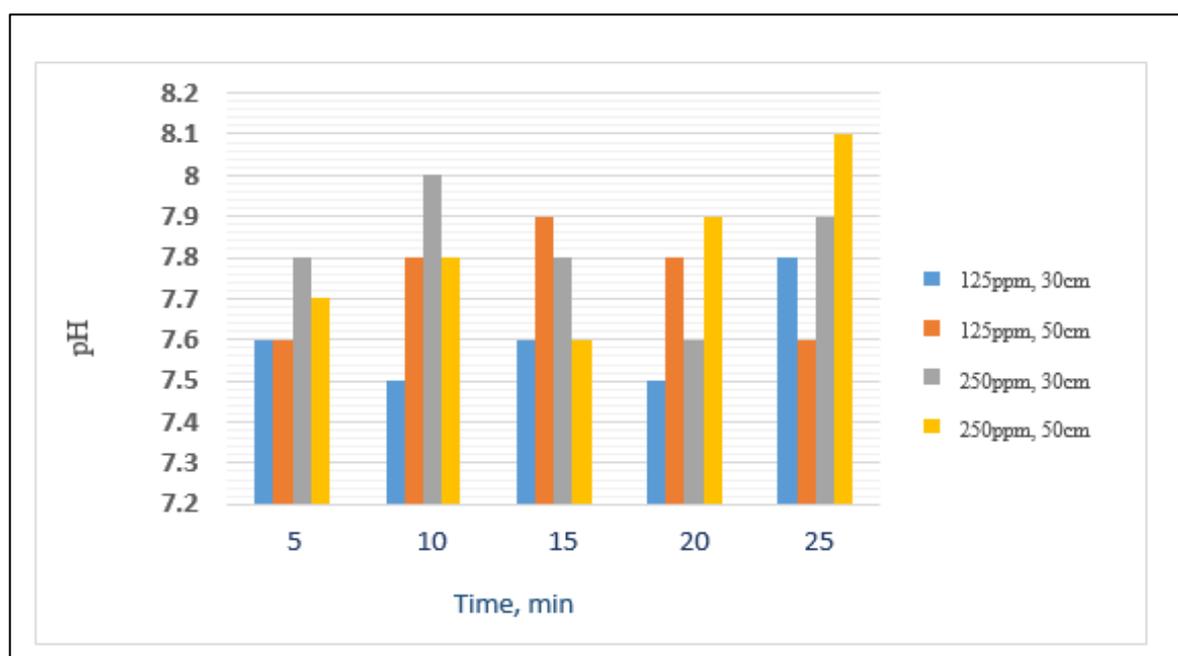


Fig. 4.19: Effluent water pH after passing through pipes (30 and 50 cm) coated with two concentrations of AgNPs (125 and 250 ppm).

These values seemed to be rather slightly alkaline, however, they were within the permissible limits of the Iraqi standard, which ranged between 6.5 and 8.5.

In the case of water EC results, the obtained data ranged from 1380 $\mu\text{s}/\text{cm}$ after 10 min in effluent water flowing via 30 cm pipes coated with 250 ppm to 1391 $\mu\text{s}/\text{cm}$ after 25 min from effluent water passing via 50 cm pipes coated with 125 ppm (Fig. 4.20).

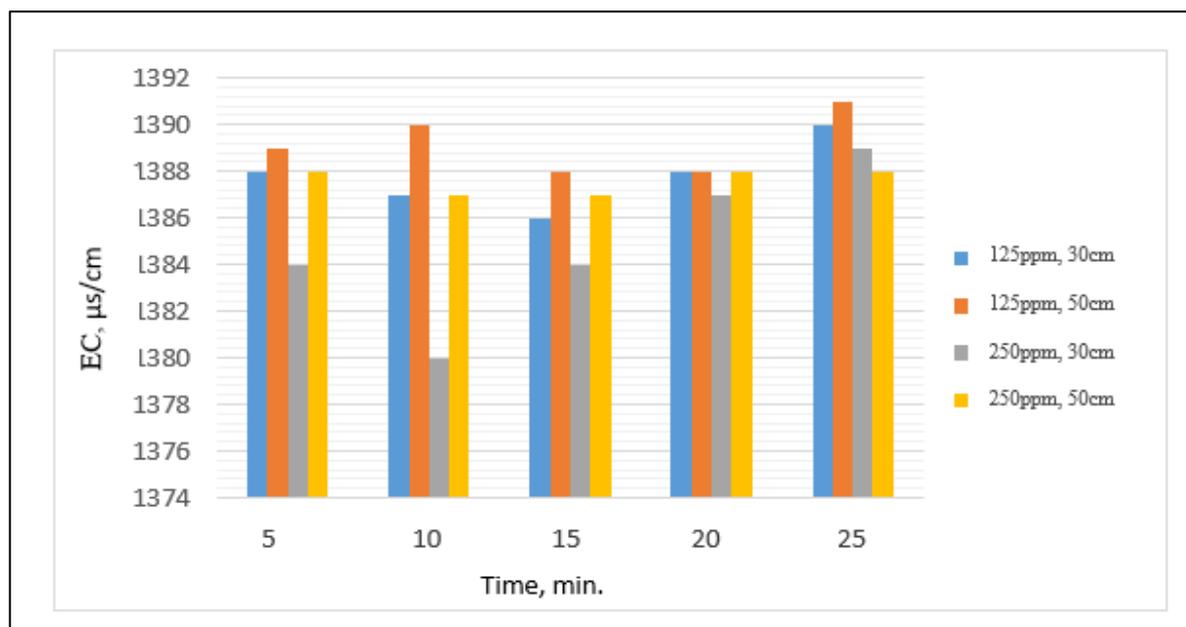


Fig. 4.20: Effluent water electrical conductivity (EC) after passing through pipes (30 and 50 cm) coated with two concentrations of AgNPs (125 and 250 ppm).

The values of TDS were not exceeded 836 mg/L where they ranged from minimum value of 828 mg/L recorded after 10 min in effluent water passing through pipes of 50 cm length coated by 250 ppm to a maximum value of 836 mg/L after 5 min in effluent water passing via 50 cm pipes coated by 125 ppm (Fig. 4.21).

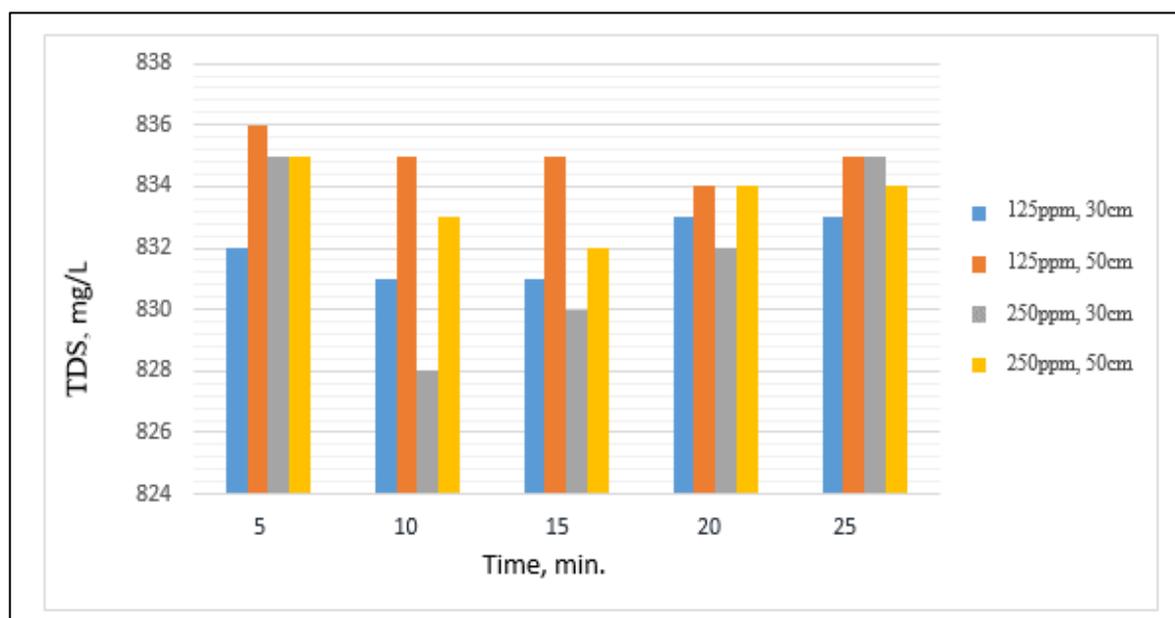


Fig. 4.21: Total dissolved solids (TDS) in effluent water after passing through pipes (30 and 50 cm) coated with two concentrations of AgNPs (125 and 250 ppm).

Obviously, these recorded results were within the permissible limits of the Iraqi drinking water standard (≤ 1000 mg/L).

4.6 Analysis of Silver Released in the Effluent Water

Due to possible human health effects of silver exposure, the silver water content was analyzed by Atomic Absorption Spectrophotometer (AAS), where the water flow via both 30 and 50 cm water pipes and subjected to AgNPs concentration of 250 ppm was examined for possible silver content.

Apparently, AAS testing of the water exposed to AgNPs concentration has shown that water silver contents varied from undetectable value up to 0.001 ppm (Table 4.5). These results have indicated that there was no elevated silver concentration in effluent water and had certain health effects in which the permissible silver content in drinking water should be less than 0.1 ppm as suggested by the US-EPA guideline for drinking water (EPA, 2018).

Table 4.5: Measurement of Ag content by AAS.

Time (min)	Ag content, ppm Pipe coated with AgNPs of 250 ppm	
	Pipe length, 30 cm	Pipe length, 50 cm
2	N/D	N/D
5	N/D	N/D
10	N/D	N/D
15	0.001	N/D
20	0.001	0.001
25	0.001	0.001
30	0.001	0.001

4.7 Cytotoxic of AgNPs by MTT Assay

4.7.1 MTT Assay

Practically, the MTT assay is an important bio-indicator for the inspection of many kinds of chemical and biological factors because of their sensitivity for measuring the impact on their recurrence rate (Bezza et al., 2020). Because AgNPs

have special properties such as distinct surface area, high reaction activity, and high penetration influences, they are employed in different fields, such as industrial, medical, environmental, and pharmaceutical applications. Accordingly, AgNPs have gained access to daily life, and the inevitable human exposure to these nanoparticles has raised concerns about their potential health hazards, where the toxicity issues for AgNPs have become an important and inevitable requirement. The MTT assay was performed to measure the cytotoxicity of AgNPs on white blood cells (WBCs), table (4.6) showed the absorbance value of Formazan formation as a cell activity indicator at 500 nm. From these results, it seems that there are no significant differences between tested and control samples at $P \leq 0.05$ where the cytotoxic effects of AgNPs on WBCs by MTT assay at microtiter plate are presented in Fig. (4.19) at the different exposure times (5, 10, 15, 20, and 25 min) and at two different types of exposure manner (produced water and direct exposure).

When the inhibition rate was calculated, the highest Inhibition value was 2.68% at 250 ppm after (15, 20, and 25 min) at direct exposure manner as shown in Figs. (4.20) and (4.21).

From these results, it can be concluded that AgNPs were safe and nontoxic even in direct exposure and can be considered a promising lining material in the manufacture of water distribution pipes. The toxic mechanisms were not observed at AgNPs, and they did not show high toxic rates on WBCs, because they did not activate the apoptosis pathways associated with the p53 gene.

Table 4.6: Cytotoxic effects of AgNPs prepared by the green synthesis with MTT assay on WBCs.

Concentration, ppm	Absorbency values in the case of produced water									
	30 cm, pipe length					50 cm, pipe length				
	5 min	10 min	15 min	20 min	25 min	5 min	10 min	15 min	20 min	25 min
0.0 (control)	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01
125	1.85 ±0.02	1.85 ±0.01	1.85 ±0.01	1.85 ±0.04	1.84 ±0.03	1.84 ±0.02	1.84 ±0.03	1.84 ±0.08	1.84 ±0.01	1.84 ±0.08
250	1.84 ±0.03	1.84 ±0.02	1.84 ±0.08	1.84 ±0.06	1.84 ±0.05	1.84 ±0.08	1.84 ±0.05	1.84± 0.02	1.84 ±0.02	1.84 ±0.04
P value=0.027										
Concentration, ppm	Absorbency values in the case of direct exposure									
	5 min	10 min	15 min	20 min	25 min	5 min	10 min	15 min	20 min	25 min
	5 min	10 min	15 min	20 min	25 min	5 min	10 min	15 min	20 min	25 min
0.0 (control)	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01	1.86 ±0.01
125	1.85 ±0.07	1.85 ±0.01	1.85 ±0.01	1.85 ±0.06	1.85 ±0.05	1.84 ±0.01	1.84 ±0.01	1.84 ±0.02	1.84 ±0.07	1.82 ±0.01
250	1.84 ±0.01	1.84 ±0.04	1.84 ±0.02	1.82 ±0.01	1.82 ±0.03	1.82 ±0.01	1.82 ±0.04	1.81 ±0.04	1.81 ±0.02	1.81 ±0.04
P value=0.023										

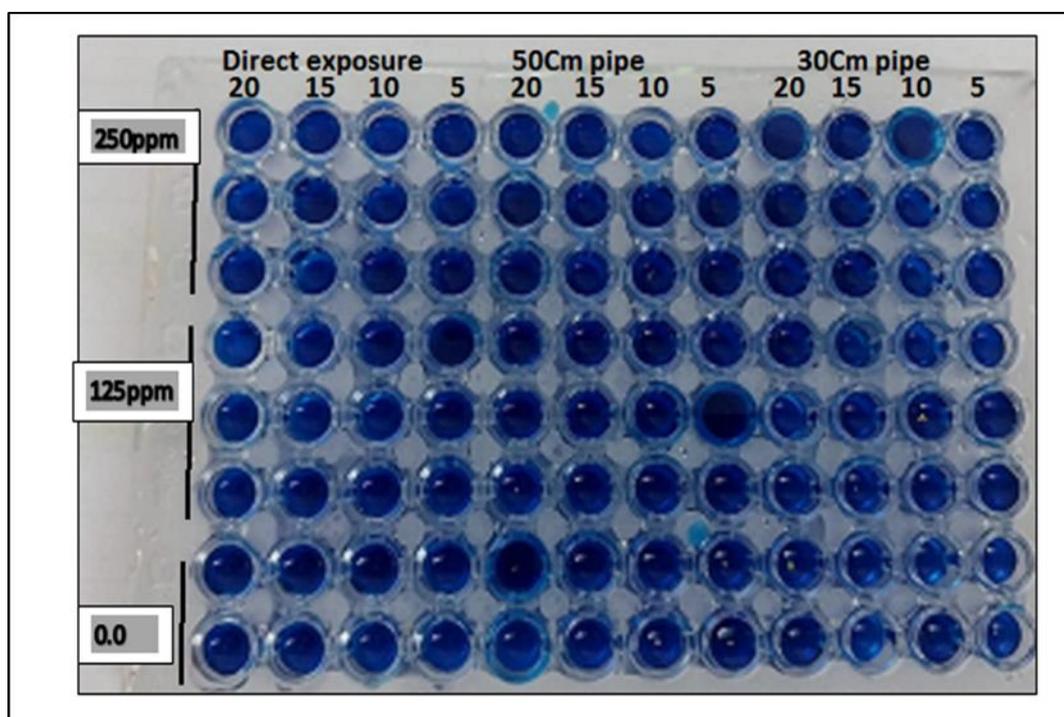


Fig. 4.19: Cytotoxic effects of AgNPs on WBCs by MTT assay at microtiter plate.

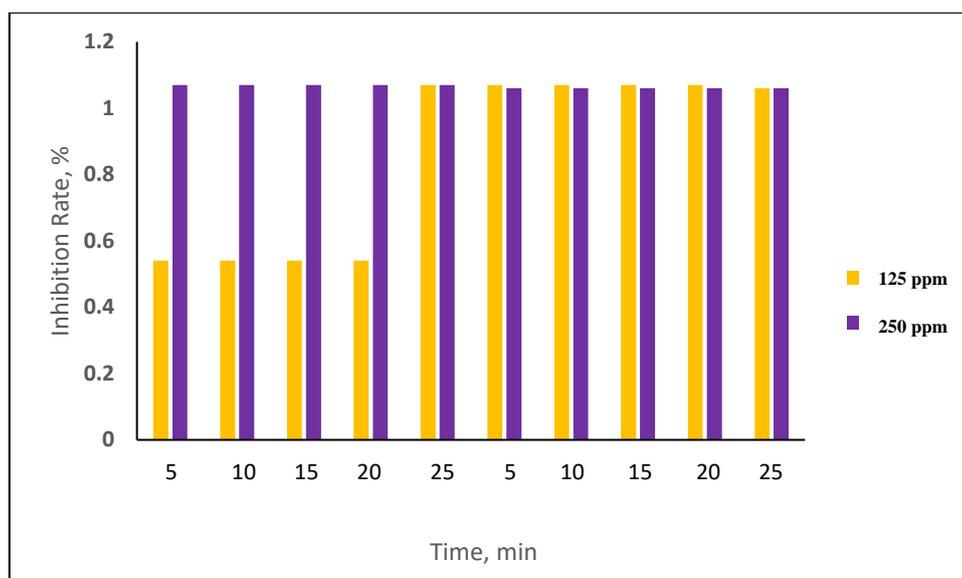


Fig. 4.20: Inhibition rate of WBSs exposed to different concentrations of AgNPs at different exposure time (produced water).

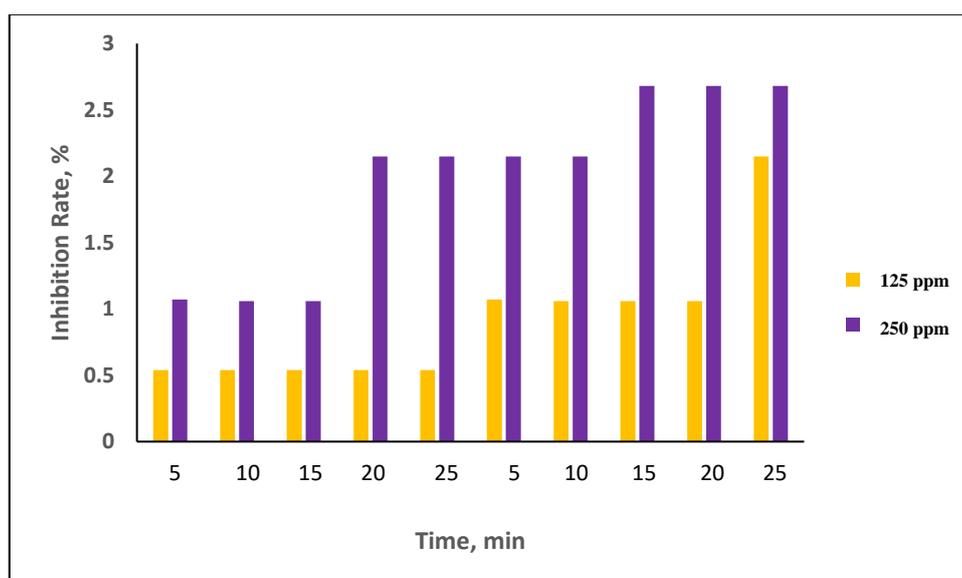


Fig. 4.21: Inhibition rate of WBSs exposed to different concentrations of AgNPs at different exposure times (direct exposure).

4.8 Hemocompatibility of AgNPs with Red Blood Cells (RBCs)

Calculation of hemocompatibility is a considerable methods for measuring different material compatibility with various types of cells and tissues as shown in Fig. (4.22). The results have shown that AgNPs did not cause significant ($P \leq 0.05$)

hemolysis in the exposed blood samples where the highest hemolysis rate was 1.78% in 250 ppm after 25 min at both exposure manners (direct and produce water) but it had no significant differences. So, this is another experimental confirmation of the non-toxicity of AgNPs on the blood cells of humans. According to ASTM F756-00 (2000) standard, materials with a hemolysis percentage $>5\%$ are considered hemolytic, whereas those between 5% and 2% were classified as slightly hemolytic. On the other hand, if the material has a hemolysis percentage $<2\%$, it is considered to be a nonhemolytic material (Fazley et al., 2014).

Any material related to human health and safety should have low blood reactivity and low hemolytic effects with high biocompatibility. Therefore, it was very important to test the prepared AgNPs at two concentrations 125 and 250 ppm as shown in Table 4.7, Fig. (4.23) and Fig. (4.24). The hemolysis assay RBCs can be considered as fragile cells being contains phospholipids in their cell membranes with high concentrations of both oxygen and hemoglobin. The impact of AgNPs was found to be related to several processes such as membrane maintenance, lipids and proteins alteration of the cell membrane that causes loss in cell water content by osmosis phenomenon. Foreign materials enforce the red blood cell (erythrocytes) to alter their form due to the asymmetrical extent of the red cell membrane's two monolayers.

Once the nanoparticles move into the internal layer, they form stomatocytes, while in case of reaching the outer moiety, they result in speculated-shaped echinocytes. Apparently, the same cause was documented by previous studies (McConkey et al., 1998; Bezza et al., 2020). The case of AgNPs mechanism hasn't been achieved and the RBCs remained cell membrane stable. So it is necessary to conduct toxicity and biocompatibility tests before applying the nanoparticles in different fields, especially, related to human health.

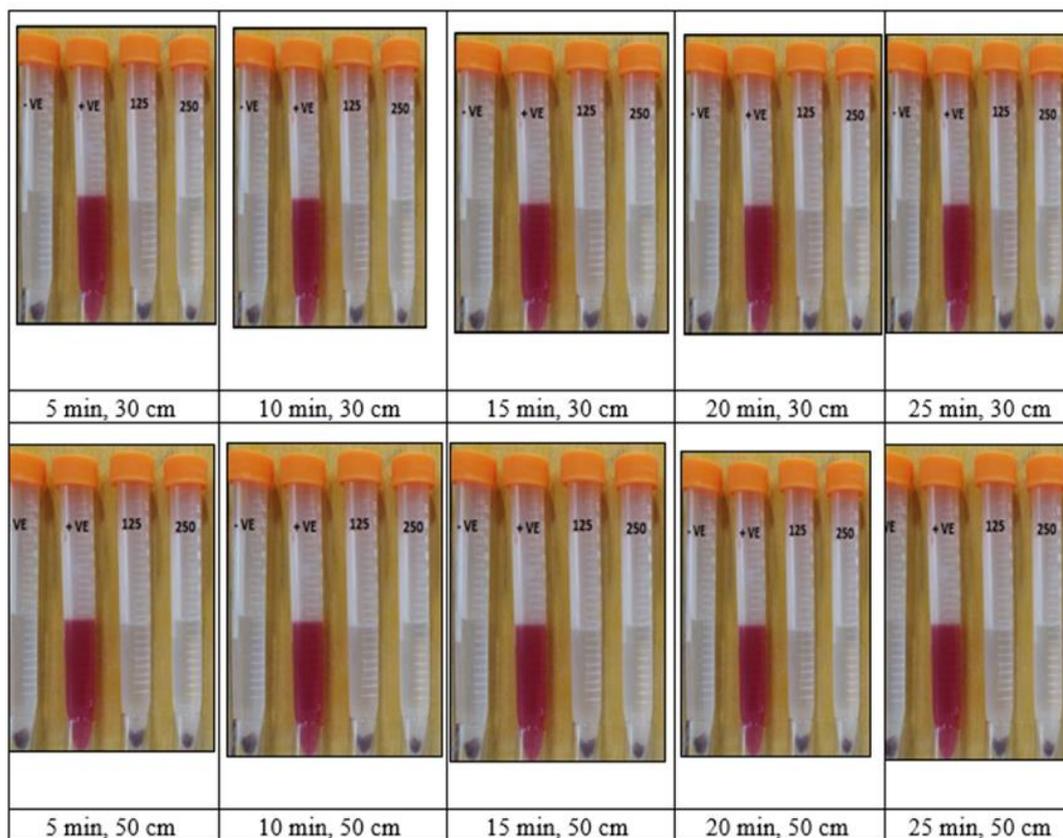


Fig. 4.22: Hemolysis Assay.

Table 4.7: Hemocompatibility assay of different concentrations of AgNPs prepared by the green synthesis on RBCs

Concentration ppm	Absorbency values in the case of produced water (OD) 542 nm									
	30 cm, pipe length					50 cm, pipe length				
	5 min	10 min	15 min	20 min	25 min	5 min	10 min	15 min	20 min	25 min
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
125	0.006	0.006	0.006	0.007	0.007	0.006	0.007	0.008	0.009	0.01
250	0.007	0.008	0.008	0.009	0.009	0.008	0.008	0.009	0.01	0.012
Positive Control	0.674									
Concentration ppm	Absorbency values in the case of direct exposure (OD) 542nm									
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	125	0.006	0.006	0.006	0.007	0.007	0.006	0.007	0.008	0.009
250	0.007	0.008	0.008	0.009	0.009	0.008	0.008	0.009	0.01	0.012
Positive Control	0.674									

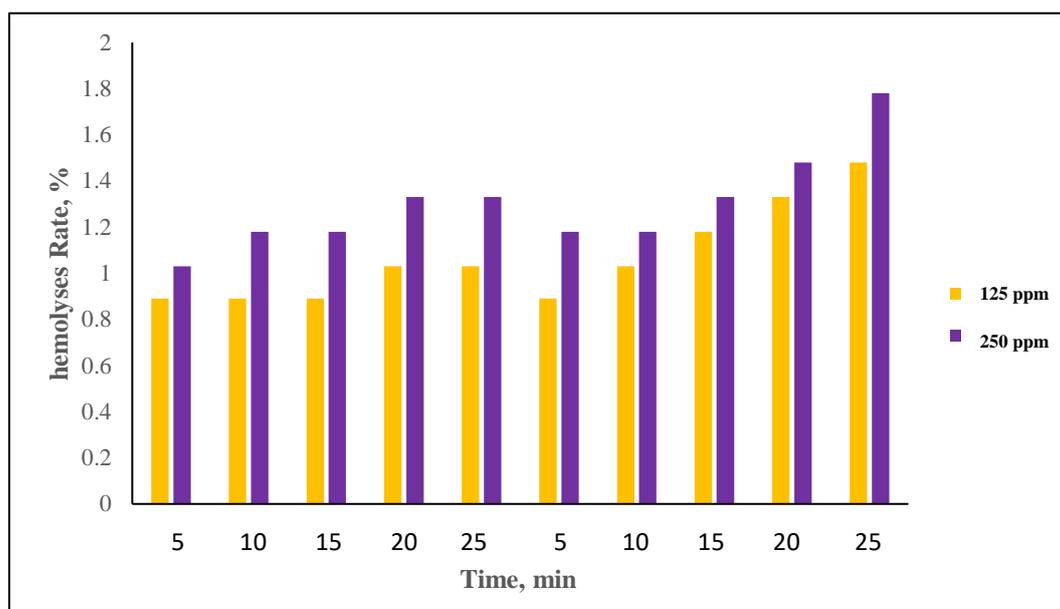


Fig. 4.23: Hemolysis rate (%) of RBCs exposed to different concentrations of AgNPs synthesized by the green methods (produced water).

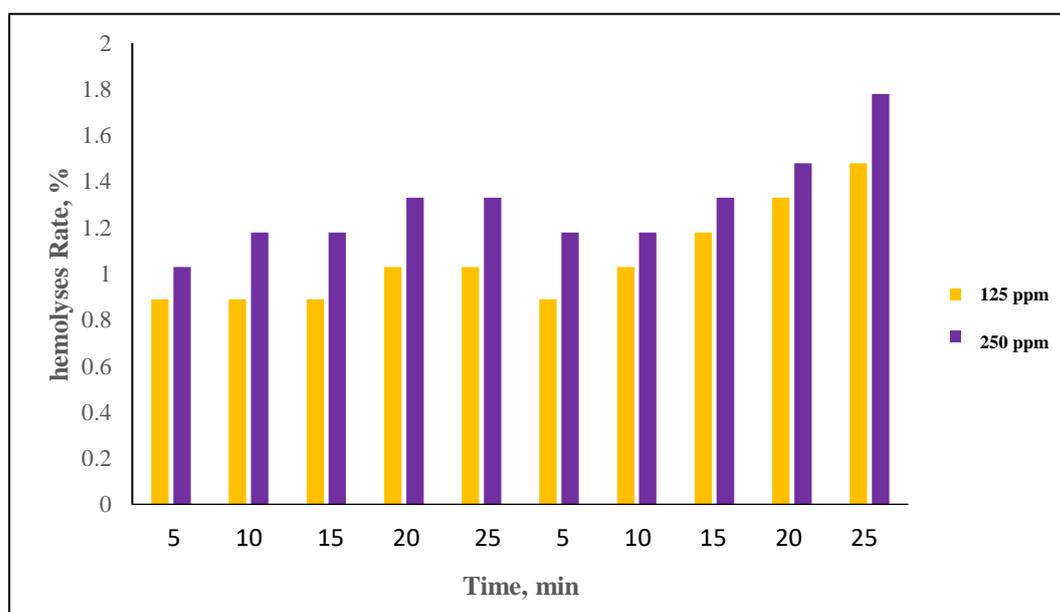
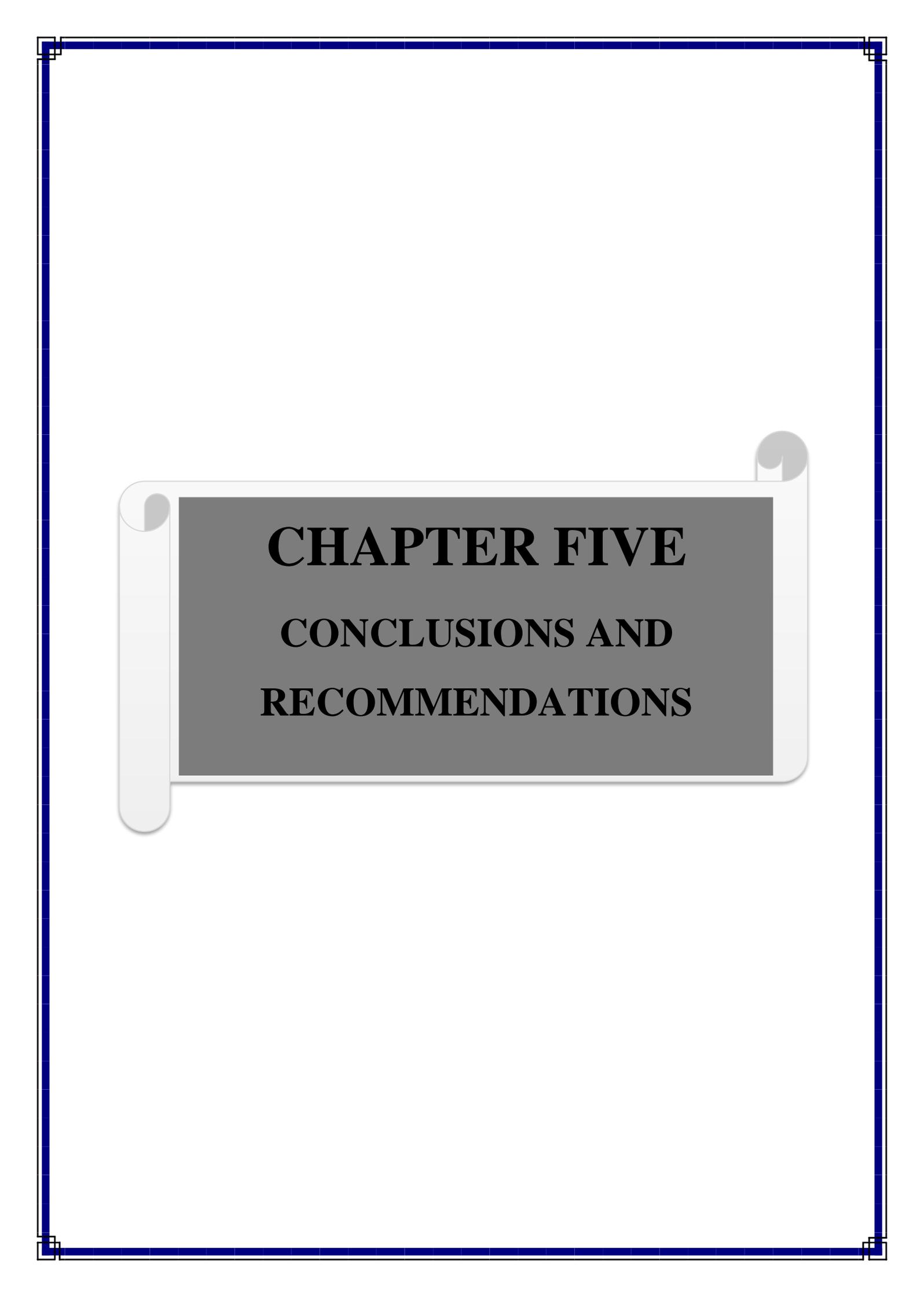


Fig. 4.24: Hemolysis rate (%) of RBCs exposed to different concentrations of AgNPs synthesized by the green methods (direct exposure).



CHAPTER FIVE
CONCLUSIONS AND
RECOMMENDATIONS

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

In this chapter, several results from the current study can be obviously given. Furthermore, it covers certain ideas and thoughts that can be worth examining through future works.

5.2 Conclusions

Depending on the current study's obtained data, several results can be withdrawn and highlighted which are:

1. Silver nanoparticles were successfully prepared from the aqueous extract of *Azadirachta indica* leaves that acted as natural weak reduction agents in addition to their roles as dispersants in preventing the aggregation of silver nanoparticles and turning them into micro aggregates. Also, they contain natural substances that have can inhibit the growth of bacteria where they remain stable on the surface of synthesized silver nanoparticles.
2. There was significant relation between silver nanoparticles' size and shape with their impact on water coliform, where the little size and globular shape of the silver nanoparticles as well as the large surface area showed a good antibacterial influence against gram-negative bacteria.
3. The current work data have shown that AgNPs coating had obvious influences against both water *E. coli* and *P. aeruginosa*, where the inhibition rate of 100% was recorded beyond a few minutes of passing the bacteria-laden water via PVC pipes coated with silver nanoparticles, since the exposure time and concentration of AgNps have direct effect on the rate of bacterial inhibition.

4. The amount of silver released from the coated water pipes was almost very small, and in certain cases, it was undetected. So the silver concentration in drinking water has complied with the Environmental Protection Agency (EPA) and World Health Organization (WHO) drinking water standards that should not exceed 0.1 mg/L, these results may indicate that the preparation of silver nanoparticles by the the green methods has improved the retention of silver and longevity of the coating.
5. Some water quality parameters such as pH, TDS, and EC were not changed after drinking water passed through AgNPs-coated PVC pipes.
6. In the cytotoxic test by MTT assay, it can be concluded that AgNPs were safe and nontoxic even in the case of direct exposure. Where it can be considered as a promising lining material in manufacturing water distribution pipes since AgNPs have not shown high toxic rates on WBCs, because they did not activate the apoptosis pathways associated with the p53gene.
7. It seems obvious that AgNPs did not cause a significant ($P \leq 0.05$) hemolysis in the exposed blood samples. The highest found hemolysis rate was small rate at both exposure types (direct and produced water). So this may be further experimental confirmation of the non-toxicity of these concentrations of AgNPs on human blood cells.

5.3 Recommendations

5.3.1 Contribution to Knowledge

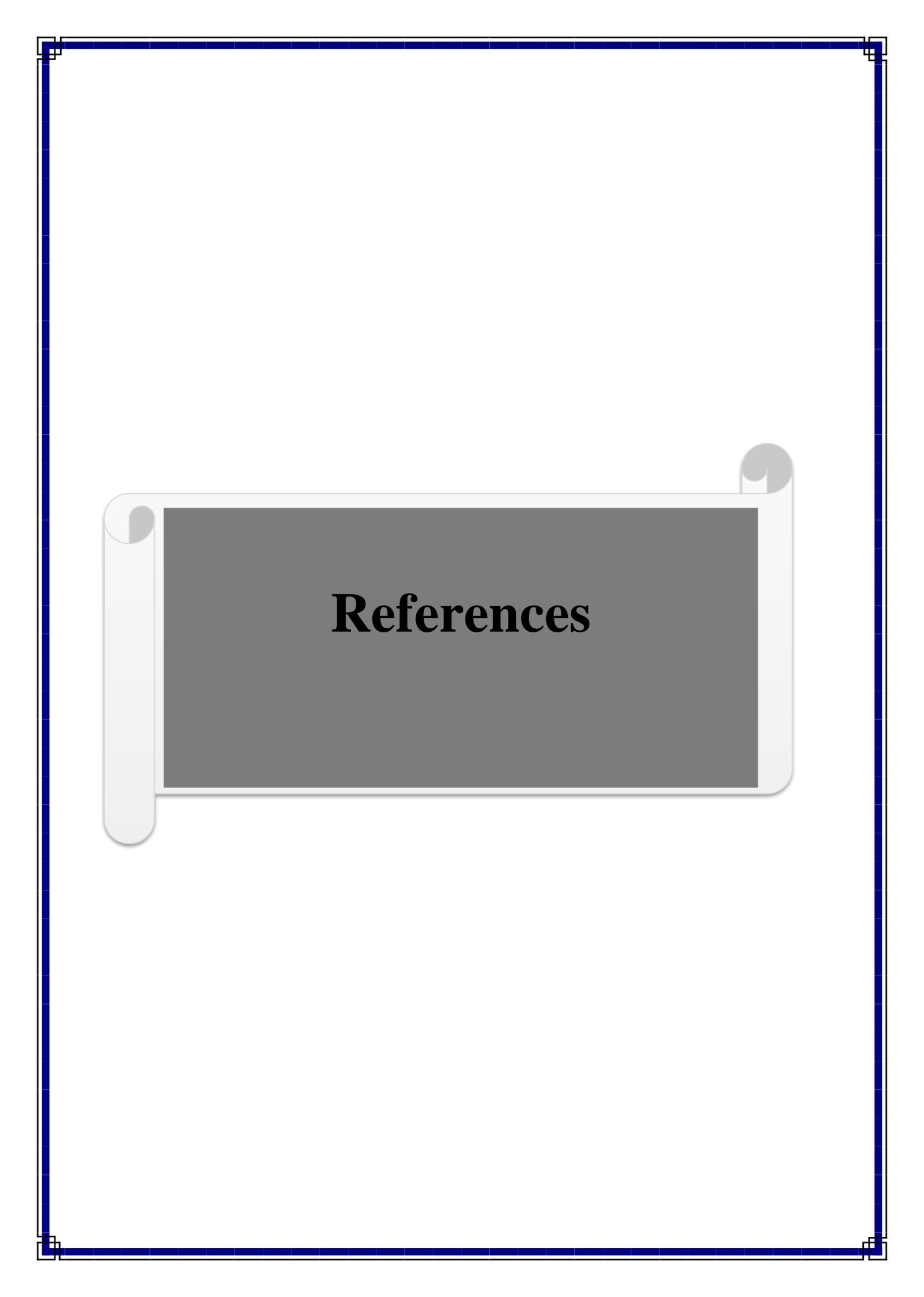
1. It is necessary to intensify efforts and broaden the horizon of cooperation between state institutions concerned with the task of improving the quality of drinking water and benefit of global expertise in the field of nanotechnology and make it an alternative to the traditional methodss used in disinfection of drinking water due to the cumulative health risks that the current methodss carry by releasing of disinfection by-products (DBPs).

2. Strengthening the applicable control systems, developing legislation and laws in force, and building the capacities of workers in the field of improving drinking water and eliminating diseases transmitted through drinking water, using modern methods and advanced technologies in controlling and preventing the spread of diseases transmitted through drinking water.

5.3.2 Recommendation for Future Works

For future works, the current work presents the following to be examined and studied as further works:

1. Using AgNPs in other concentrations in order to determine the optimal concentration for the nano-coating as an anti-bacterial agent.
2. Studying the use of innovative coating methods for coating water pipes to ensure the best efficiency and lowest manufacturing cost and study the effect of high water temperature on coating efficiency.
3. More Studies about the effectiveness of AgNPs in microbial inactivation by testing the other types of pathogenic bacteria common in drinking water.
4. Further studies on the long-term working efficiency of AgNPs- coated pipes.
5. Studying the efficiency of silver nanoparticles in coating water pipes in homes that deliver drinking water from the tank to domestic consumption, or coating drinking pipes in health institutions.
6. Developing a feasibility study to find out the economic cost of the silver nanoparticles coating process.



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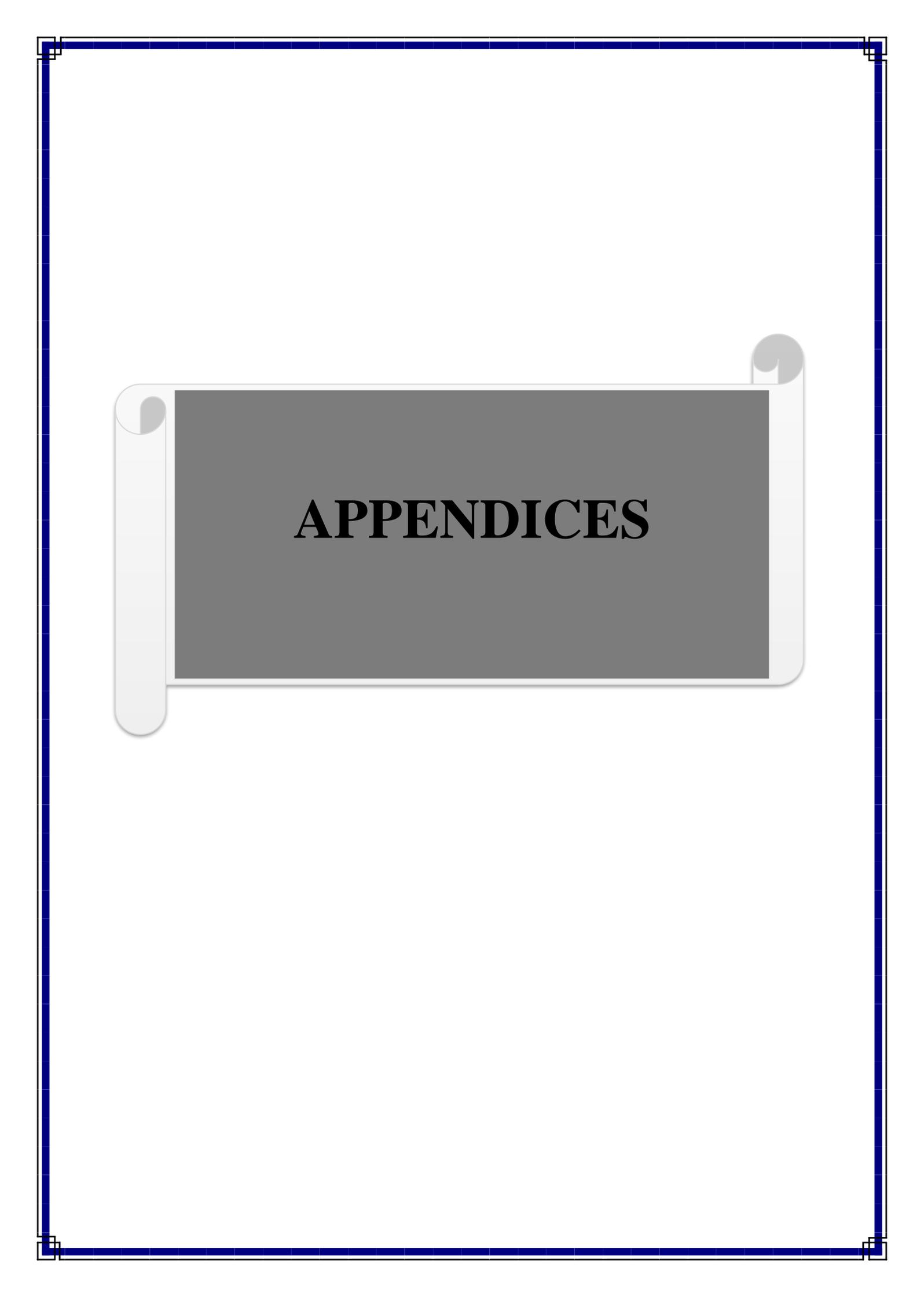
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APPENDICES

APPENDIX – A



Fig. A.1: X-Ray Diffraction.



Fig. A.2: Field Emission
Scanning Electron Microscopy (FE-SEM)



Fig. A.3: Transmission Electron Microscopy (TEM).



Fig. A.4: Biological safety cabinet.



Fig. A.5: Atomic Absorption Spectrometry (AAS).

DRINKING WATER GUIDELINES

Table A.1: World Health Organization Guideline values for bacteriological quality.
(2011)

Organisms	Guideline value
All water intended for drinking	
<i>E. coli</i> or thermotolerant coliform bacteria ^{b,c}	Must not be detectable in any 100-ml sample
Treated water entering the distribution system	
<i>E. coli</i> or thermotolerant coliform bacteria ^b	Must not be detectable in any 100-ml sample
Total coliform bacteria	Must not be detectable in any 100-ml sample
Treated water in the distribution system	
<i>E. coli</i> or thermotolerant coliform bacteria ^b	Must not be detectable in any 100-ml sample
Total coliform bacteria	Must not be detectable in any 100-ml sample. In the case of large supplies, where sufficient samples are examined, must not be present in 95% of samples taken throughout any 12-month period

Table A.2: US Environmental Protection Agency Drinking Water Standards and Health Advisories.

EPA, 2000

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories						Cancer Descriptor ¹
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		10-kg Child		RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10 ⁻⁴ Cancer Risk	
						One-day (mg/L)	Ten-day (mg/L)					
ORGANICS												
Acenaphthene	83-32-9	-	-	-	-	-	-	0.06	2	-	-	-
Acifluorfen (sodium)	62476-59-9	-	-	-	F '88	2	2	0.01	0.4	-	0.1	L/N
Acrylamide	79-06-1	F	zero	TT ²	F '87	1.5	0.3	0.002	0.07	-	-	L
Acrylonitrile	107-13-1	-	-	-	-	-	-	-	-	-	0.006	B1
Alachlor	15972-60-8	F	zero	0.002	F '88	0.1	0.1	0.01	0.4	-	0.04	B2
Aldicarb ³	116-06-3	F ⁴	0.001	0.003	F '95	0.01	0.01	0.001	0.035	0.007	-	D
Aldicarb sulfone ³	1646-88-4	F ⁴	0.001	0.002	F '95	0.01	0.01	0.001	0.035	0.007	-	D
Aldicarb sulfoxide ³	1646-87-3	F ⁴	0.001	0.004	F '95	0.01	0.01	0.001	0.035	0.007	-	D
Aldrin	309-00-2	-	-	-	F '92	0.0003	0.0003	0.00003	0.001	-	0.0002	B2
Ametryn	834-12-8	-	-	-	F '88	9	9	0.009	0.3	0.06	-	D
Ammonium sulfamate	7773-06-0	-	-	-	F '88	20	20	0.2	8	2	-	D
Anthracene (PAH) ⁵	120-12-7	-	-	-	-	-	-	0.3	10	-	-	D
Atrazine	1912-24-9	F	0.003	0.003	F '88	-	-	0.02	0.7	-	-	N
Baygon	114-26-1	-	-	-	F '88	0.04	0.04	0.004	0.1	0.003	-	C
Bentazon	25057-89-0	-	-	-	F '99	0.3	0.3	0.03	1	0.2	-	E
Benz(a)anthracene (PAH)	56-55-3	-	-	-	-	-	-	-	-	-	-	B2
Benzene	71-43-2	F	zero	0.005	F '87	0.2	0.2	0.004	0.1	0.003	1 to 10	H
Benzo(a)pyrene (PAH)	50-32-8	F	zero	0.0002	-	-	-	-	-	-	0.0005	B2
Benzo(b)fluoranthene (PAH)	205-99-2	-	-	-	-	-	-	-	-	-	-	B2
Benzo(g,h,i)perylene (PAH)	191-24-2	-	-	-	-	-	-	-	-	-	-	D
Benzo(k)fluoranthene (PAH)	207-08-9	-	-	-	-	-	-	-	-	-	-	B2
Bis(2-chloro-1-methylethyl) ether	108-60-1	-	-	-	F '89	4	4	0.04	1	0.3	-	-
Bromacil	314-40-9	-	-	-	F '88	5	5	0.1	3.5	0.07	-	C
Bromobenzene	108-86-1	-	-	-	D '86	4	4	0.008	0.3	0.06	-	I

¹Chemicals evaluated under the 2005 Cancer Guidelines or the 1996 or 1999 drafts are demoted by an abbreviation for their weight-of-the-evidence descriptor (see page iii). If the agency has not completed a new assessment for the chemical, the 1986 Guidelines Group designation (see page iii) is given in the Cancer Descriptor column

²When Acrylamide is used in drinking water systems, the combination (or product) of dose and monomer level shall not exceed that equivalent to a polyacrylamide polymer containing 0.05% monomer dosed at 1 mg/L

³The MCL value for any combination of two or more of these three chemicals should not exceed 0.007 mg/L because of a similar mode of action.

⁴Administrative stay of the effective date.

⁵PAH = Polycyclic aromatic hydrocarbon.

continuing

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories						Cancer Descriptor
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		10-kg Child		RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10 ⁻⁴ Cancer Risk	
						One-day (mg/L)	Ten-day (mg/L)					
Bromochloromethane	74-97-5	-	-	-	F '89	50	1	0.01	0.5	0.09	-	D
Bromodichloromethane (THM)	75-27-4	F	zero	0.08 ¹	-	1	0.6	0.003	0.1	-	0.1	L
Bromoform (THM)	75-25-2	F	zero	0.08 ¹	-	5	0.2	0.03	1	-	0.8	L
Bromomethane	74-83-9	-	-	-	D '89	0.1	0.1	0.001	0.05	0.01	-	D
Butyl benzyl phthalate	85-68-7	-	-	-	-	-	-	0.2	7	-	-	C
Butylate	2008-41-5	-	-	-	F '89	2	2	0.05	2	0.4	-	D
Carbaryl	63-25-2	-	-	-	F '88	1	1	0.01	0.4	-	4	L
Carbofuran	1563-66-2	F	0.04	0.04	F '87	-	-	0.00006	-	-	-	N
Carbon tetrachloride	56-23-5	F	zero	0.005	F '87	4	0.2	0.004	0.1	0.03	0.05	L
Carboxin	5234-68-4	-	-	-	F '88	1	1	0.1	3.5	0.7	-	D
Chloramben	133-90-4	-	-	-	F '88	3	3	0.015	0.5	0.1	-	D
Chlordane	12798-03-6	F	zero	0.002	F '87	0.06	0.06	0.0005	0.02	0.004	0.01	B2
Chloroform (THM)	67-66-3	F	0.07	0.08 ¹	-	4	4	0.01	0.35	0.07	-	L/N
Chloromethane	74-87-3	-	-	-	F '89	9	0.4	-	-	-	-	I
Chlorophenol (2-)	95-57-8	-	-	-	D '94	0.5	0.5	0.005	0.2	0.04	-	D
Chlorothalonil	1897-45-6	-	-	-	F '88	0.2	0.2	0.015	0.5	-	0.15	B2
Chlorotoluene o-	95-49-8	-	-	-	F '89	2	2	0.02	0.7	0.1	-	D
Chlorotoluene p-	106-43-4	-	-	-	F '89	2	2	0.02	0.7	0.1	-	D
Chlorpyrifos	2921-88-2	-	-	-	F '92	0.03	0.03	0.0003	0.01	0.002	-	D
Chrysene (PAH)	218-01-9	-	-	-	-	-	-	-	-	-	-	B2
Cyanazine	21725-46-2	-	-	-	D '96	0.1	0.1	0.002	0.07	0.001	-	

¹1998 Final Rule for Disinfectants and Disinfection By-products: The total for trihalomethanes (THM) is 0.08 mg/L

continuing

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories						Cancer Descriptor
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		10-kg Child		RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10 ⁻⁴ Cancer Risk	
						One-day (mg/L)	Ten-day (mg/L)					
Cyanogen chloride ¹	506-77-4	-	-	-	-	0.05	0.05	0.05	2	-	-	D
2,4-D (2,4-dichlorophenoxyacetic acid)	94-75-7	F	0.07	0.07	F '87	1	0.3	0.005	0.2	-	-	D
DCPA (Dacthal)	1861-32-1	-	-	-	F '08	2	2	0.01	0.35	0.07	-	C
Dalapon (sodium salt)	75-99-0	F	0.2	0.2	F '89	3	3	0.03	0.9	0.2	-	D
Di(2-ethylhexyl)adipate	103-23-1	F	0.4	0.4	-	20	20	0.6	20	0.4	3	C
Di(2-ethylhexyl)phthalate	117-81-7	F	zero	0.006	-	-	-	0.02	0.7	-	0.3	B2
Diazinon	333-41-5	-	-	-	F '88	0.02	0.02	0.0002	0.007	0.001	-	E
Dibromochloromethane (THM)	124-48-1	F	0.06	0.08 ²	-	0.6	0.6	0.02	0.7	0.06	0.08	S
Dibromochloropropane (DBCP)	96-12-8	F	zero	0.0002	F '87	0.2	0.05	-	-	-	0.003	B2
Dibutyl phthalate	84-74-2	-	-	-	-	-	-	0.1	4	-	-	D
Dicamba	1918-00-9	-	-	-	F '88	-	-	0.5	18	4	-	N
Dichloroacetic acid	76-43-6	F	zero	0.06 ³	-	3	3	0.004	0.1	0.03	0.07	L
Dichlorobenzene o-	95-50-1	F	0.6	0.6	F '87	9	9	0.09	3	0.6	-	D
Dichlorobenzene — ⁴	541-73-1	-	-	-	F '87	9	9	0.09	3	0.6	-	D
Dichlorobenzene p-	106-46-7	F	0.075	0.075	F '87	11	11	0.1	4	0.075	-	C
Dichlorodifluoromethane	75-71-8	-	-	-	F '89	40	40	0.2	5	1	-	D
Dichloroethane (1,2-)	107-06-2	F	zero	0.005	F '87	0.7	0.7	-	-	-	0.04	B2
Dichloroethylene (1,1-)	75-35-4	F	0.007	0.007	F '87	2	1	0.05	2	0.4	0.006	S
Dichloroethylene (cis-1,2-)	156-59-2	F	0.07	0.07	F '90	4	3	0.002	0.07	0.01	-	I
Dichloroethylene (trans-1,2-)	156-60-5	F	0.1	0.1	F '87	20	2	0.02	0.7	0.1	-	I
Dichloromethane	75-09-2	F	zero	0.005	D '93	10	2	0.06	2	0.2	0.5	L
Dichlorophenol (2,4-)	120-83-2	-	-	-	D '94	0.03	0.03	0.003	0.1	0.02	-	E
Dichloropropane (1,2-)	78-87-5	F	zero	0.005	F '87	-	0.09	-	-	-	0.06	B2
Dichloropropene (1,3-)	542-75-6	-	-	-	F '88	0.03	0.03	0.03	1	-	0.04	L
Dieldrin	60-57-1	-	-	-	F '88	0.0005	0.0005	0.00005	0.002	-	0.0002	B2
Diethyl phthalate	84-66-2	-	-	-	-	-	-	0.8	30	-	-	D

¹Under review.

²1998 Final Rule for Disinfectants and Disinfection By-products: The total for trihalomethanes is 0.08 mg/L.

³1998 Final Rule for Disinfectants and Disinfection By-products: The total for five haloacetic acids is 0.06 mg/L.

⁴The values for m-dichlorobenzene are based on data for o-dichlorobenzene

continuing

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories						Cancer Descriptor
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		10-kg Child		RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10 ⁻⁴ Cancer Risk	
						One-day (mg/L)	Ten-day (mg/L)					
Diisopropylmethylphosphonate	1445-75-6	-	-	-	F '89	8	8	0.08	3	0.6	-	D
Dimethrin	70-38-2	-	-	-	F '88	10	10	0.3	10	2	-	D
Dimethyl methylphosphonate	756-79-6	-	-	-	F '92	2	2	0.2	7	0.1	0.7	C
Dimethyl phthalate	131-11-3	-	-	-	-	-	-	-	-	-	-	D
Dinitrobenzene (1,3-)	99-65-0	-	-	-	F '91	0.04	0.04	0.0001	0.005	0.001	-	D
Dinitrotoluene (2,4-)	121-14-2	-	-	-	F '08	1	1	0.002	0.1	-	0.005	L
Dinitrotoluene (2,6-)	606-20-2	-	-	-	F '08	0.4	0.04	0.001	0.04	-	0.005	L
Dinitrotoluene (2,6 & 2,4) ¹		-	-	-	F '92	-	-	-	-	-	0.005	B2
Dinoseb	88-85-7	F	0.007	0.007	F '88	0.3	0.3	0.001	0.035	0.007	-	D
Dioxane p-	123-91-1	-	-	-	F '87	4	0.4	0.03	1	0.2	0.035	L
Diphenamid	957-51-7	-	-	-	F '88	0.3	0.3	0.03	1	0.2	-	D
Diquat	85-00-7	F	0.02	0.02	-	-	-	0.005	0.02	-	-	E
Disulfoton	298-04-4	-	-	-	F '88	0.01	0.01	0.0001	0.0035	0.0007	-	E
Dithiane (1,4-)	505-29-3	-	-	-	F '92	0.4	0.4	0.01	0.4	0.08	-	D
Diuron	330-54-1	-	-	-	F '88	1	1	0.003	0.1	-	0.2	L
Endothall	145-73-3	F	0.1	0.1	F '88	0.8	0.8	0.007	0.25	0.05	-	N
Endrin	72-20-8	F	0.002	0.002	F '87	0.02	0.005	0.0003	0.01	0.002	-	I
Epichlorohydrin	106-89-8	F	zero	TT ²	F '87	0.1	0.1	0.002	0.07	-	0.3	B2
Ethylbenzene	100-41-4	F	0.7	0.7	F '87	30	3	0.1	3	0.7	-	D
Ethylene dibromide (EDB) ³	106-93-4	F	zero	0.00005	F '87	0.008	0.008	0.009	0.3	-	0.002	L
Ethylene glycol	107-21-1	-	-	-	F '87	20	6	2	70	14	-	D
Ethylene Thiourea (ETU)	96-45-7	-	-	-	F '88	0.3	0.3	0.0002	0.007	-	0.06	B2
Fenamiphos	22224-92-6	-	-	-	F '88	0.009	0.009	0.0001	0.0035	0.0007	-	E

¹Technical grade.

²When epichlorohydrin is used in drinking water systems, the combination (or product) of dose and monomer level shall not exceed that equivalent to an epichlorohydrin-based polymer containing 0.01% monomer dosed at 20 mg/L.

³dibromoethane

continuing

Chemicals	CAS Number	Standards			Status HA Standards	Health Advisories						Cancer Descriptor
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		10-kg Child		RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10 ⁻⁴ Cancer Risk	
						One-day (mg/L)	Ten-day (mg/L)					
Fluometuron	2164-17-2	-	-	-	F '88	2	2	0.01	0.5	0.09	-	D
Fluorene (PAH)	86-73-7	-	-	-	-	-	-	0.04	1	-	-	D
Fonofos	944-22-9	-	-	-	F '88	0.02	0.02	0.002	0.07	0.01	-	D
Formaldehyde	50-00-0	-	-	-	D '93	10	5	0.2	7	1	-	B1 ¹
Glyphosate	1071-83-6	F	0.7	0.7	F '88	20	20	2	70	-	-	D
Heptachlor	76-44-8	F	zero	0.0004	F '87	0.01	0.01	0.0005	0.02	-	0.0008	B2
Heptachlor epoxide	1024-57-3	F	zero	0.0002	F '87	0.01	-	0.00001	0.0004	-	0.0004	B2
Hexachlorobenzene	118-74-1	F	zero	0.001	F '87	0.05	0.05	0.0008	0.03	-	0.002	B2
Hexachlorobutadiene ²	87-68-3	-	-	-	-	0.3	0.3	0.0003	0.01	-	0.09	L
Hexachlorocyclopentadiene	77-47-4	F	0.05	0.05	-	-	-	0.006	0.2	-	-	N
Hexachloroethane	67-72-1	-	-	-	F '91	5	5	0.001	0.04	0.001	0.3	C
Hexane (n-)	110-54-3	-	-	-	F '87	10	4	-	-	-	-	I
Hexazinone	51235-04-2	-	-	-	F '96	3	2	0.05	2	0.4	-	D
HMX ³	2691-41-0	-	-	-	F '88	5	5	0.05	2	0.4	-	D
Indeno(1,2,3,-c,d)pyrene (PAH)	193-39-5	-	-	-	-	-	-	-	-	-	-	B2
Isophorone	78-59-1	-	-	-	F '92	15	15	0.2	7	0.1	4	C
Isopropyl methylphosphonate	1832-54-8	-	-	-	F '92	30	30	0.1	3.5	0.7	-	D
Isopropylbenzene (cumene)	98-82-8	-	-	-	D '87	11	11	0.1	4	-	-	D
Lindane ⁴	58-89-9	F	0.0002	0.0002	F '87	1	1	0.005	0.2	-	-	S
Malathion	121-75-5	-	-	-	F '92	0.2	0.2	0.07	2	0.5	-	S
Maleic hydrazide	123-33-1	-	-	-	F '88	10	10	0.5	20	4	-	D
MCPA ⁵	94-74-6	-	-	-	F '88	0.1	0.1	0.004	0.14	0.03	-	N
Methomyl	16752-77-5	-	-	-	F '88	0.3	0.3	0.025	0.9	0.2	-	E
Methoxychlor	72-43-5	F	0.04	0.04	F '87	0.05	0.05	0.005	0.2	0.04	-	D
Methyl ethyl ketone	78-93-3	-	-	-	F '87	75	7.5	0.6	20	4	-	D
Methyl parathion	298-00-0	-	-	-	F '88	0.3	0.3	0.0002	0.007	0.001	-	N

¹Carcinogenicity based on inhalation exposure.

²Regulatory Determination Health Effects Support Document for Hexachlorobutadiene (http://www.epa.gov/safewater/ccl/pdfs/reg_determine1/support_cc1_hexachlorobutadiene_healtheffects.pdf).

³HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine.

⁴Lindane = γ - hexachlorocyclohexane.

⁵MCPA = 4 (chloro-2-methoxyphenoxy) acetic acid.

continuing

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories						Cancer Descriptor
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		10-kg Child		RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10 ⁻⁴ Cancer Risk	
						One-day (mg/L)	Ten-day (mg/L)					
Metolachlor	51218-45-2	-	-	-	F '88	2	2	0.1	3.5	0.7	-	C
Metribuzin	21087-64-9	-	-	-	F '88	5	5	0.01	0.35	0.07	-	D
Monochloroacetic acid	79-11-8	F	0.07	0.06 ¹	-	0.2	0.2	0.01	0.35	0.07	-	I
Monochlorobenzene	108-90-7	F	0.1	0.1	F '87	4	4	0.02	0.7	0.1	-	D
Naphthalene	91-20-3	-	-	-	F '90	0.5	0.5	0.02	0.7	0.1	-	I
Nitrocellulose ²	9004-70-0	-	-	-	F '88	-	-	-	-	-	-	-
Nitroguanidine	556-88-7	-	-	-	F '90	10	10	0.1	3.5	0.7	-	D
Nitrophenol p-	100-02-7	-	-	-	F '92	0.8	0.8	0.008	0.3	0.06	-	D
N-nitrosodimethylamine		-	-	-	-	-	-	-	-	-	0.00007	B2
Oxamyl (Vydate)	23135-22-0	F	0.2	0.2	F '05	0.01	0.01	0.001	0.035		-	N
Paraquat	1910-42-5	-	-	-	F '88	0.1	0.1	0.0045	0.2	0.03	-	E
Pentachlorophenol	87-86-5	F	zero	0.001	F '87	1	0.3	0.005	0.2	0.04	0.009	L
PFOA	335-67-1	-	-	-	F '16	-	-	2 x 10 ⁻⁵	3.7 x 10 ⁻⁴	7 x 10 ⁻⁵	5 x 10 ⁻²	S
PFOS	1763-23-1	-	-	-	F '16	-	-	2 x 10 ⁻⁵	3.7 x 10 ⁻⁴	7 x 10 ⁻⁵	-	S
Phenanthrene (PAH)	85-01-8	-	-	-	-	-	-	-	-	-	-	D
Phenol	108-95-2	-	-	-	D '92	6	6	0.3	11	2	-	D
Picloram	1918-02-1	F	0.5	0.5	F '88	20	20	0.02	0.7	-	-	D
Polychlorinated biphenyls (PCBs)	1336-36-3	F	zero	0.0005	D '93	-	-	-	-	-	0.01	B2
Prometon	1610-18-0	-	-	-	F '88	0.2	0.2	0.05	2	0.4	-	N
Pronamide	23950-58-5	-	-	-	F '88	0.8	0.8	0.08	3	-	0.1	B2
Propachlor	1918-16-7	-	-	-	F '88	0.5	0.5	0.05	2	-	0.1	L
Propazine	139-40-2	-	-	-	F '88	-	-	0.02	0.7	0.01	-	N
Propham	122-42-9	-	-	-	F '88	5	5	0.02	0.6	0.1	-	D
Pyrene (PAH)	129-00-0	-	-	-	-	-	-	0.03	-	-	-	D
RDX ³	121-82-4	-	-	-	F '88	0.1	0.1	0.003	0.1	0.002	0.03	C
Simazine	122-34-9	F	0.004	0.004	F '88	-	-	0.02	0.7	-	-	N
Styrene	100-42-5	F	0.1	0.1	F '87	20	2	0.2	7	0.1	-	C
2,4,5-T (Trichlorophenoxy-acetic acid)	93-76-5	-	-	-	F '88	0.8	0.8	0.01	0.35	0.07	-	D

¹1998 Final Rule for Disinfectants and Disinfection By-products: the total for five haloacetic acids is 0.06 mg/L.

²The Health Advisory Document for nitrocellulose does not include HA values and describes this compound as relatively nontoxic.

³RDX = hexahydro -1,3,5-trinitro-1,3,5-triazine.

continuing

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories						Cancer Descriptor
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		10-kg Child		RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10 ⁻⁴ Cancer Risk	
						One-day (mg/L)	Ten-day (mg/L)					
2,3,7,8-TCDD (Dioxin)	1746-01-6	F	zero	3E-08	F '87	1E-06	1E-07	1E-09	4E-08	-	2E-08	B2
Tebuthiuron	34014-18-1	-	-	-	F '88	3	3	0.07	2	0.5	-	D
Terbacil	5902-51-2	-	-	-	F '88	0.3	0.3	0.01	0.4	0.09	-	E
Terbufos	13071-79-9	-	-	-	F '88	0.005	0.005	0.0005	0.002	0.0004	-	D
Tetrachloroethane (1,1,1,2-)	630-20-6	-	-	-	F '89	2	2	0.03	1	0.07	0.1	C
Tetrachloroethane (1,1,2,2-)	79-34-5	-	-	-	F '08	3	3	0.01	0.4	-	0.04	L
Tetrachloroethylene ¹	127-18-4	F	zero	0.005	F '87	2	2	0.01	0.5	0.01	-	-
Tetrachloroterephthalic acid	236-79-0	-	-	-	F '08	100	100	-	-	-	-	I
Trichlorofluoromethane	75-69-4	-	-	-	F '89	7	7	0.3	10	2	-	D
Toluene	108-88-3	F	1	1	D '93	20	2	0.08	3	-	-	I
Toxaphene	8001-35-2	F	zero	0.003	F '96	0.004	0.004	0.0004	0.01	-	0.003	B2
2,4,5-TP (Silvex)	93-72-1	F	0.05	0.05	F '88	0.2	0.2	0.008	0.3	0.05	-	D
Trichloroacetic acid	76-03-9	F	0.02	0.06 ²	-	3	3	0.03	1	0.02	-	S
Trichlorobenzene (1,2,4-)	120-82-1	F	0.07	0.07	F '89	0.1	0.1	0.01	0.35	0.07	-	D
Trichlorobenzene (1,3,5-)	108-70-3	-	-	-	F '89	0.6	0.6	0.006	0.2	0.04	-	D
Trichloroethane (1,1,1-)	71-55-6	F	0.2	0.2	F '87	100	40	2	70	-	-	I
Trichloroethane (1,1,2-)	79-00-5	F	0.003	0.005	F '89	0.6	0.4	0.004	0.1	0.003	0.06	C
Trichloroethylene ¹	79-01-6	F	zero	0.005	F '87	-	-	0.007	0.2	-	0.3	B2
Trichlorophenol (2,4,6-)	88-06-2	-	-	-	D '94	0.03	0.03	0.0003	0.01	-	0.3	B2
Trichloropropane (1,2,3-)	96-18-4	-	-	-	F '89	0.6	0.6	0.004	0.1	-	-	L
Trifluralin	1582-09-8	-	-	-	F '90	0.08	0.08	0.02	0.7	0.01	0.4	C
Trimethylbenzene (1,2,4-)	95-63-6	-	-	-	D '87	-	-	-	-	-	-	D
Trimethylbenzene (1,3,5-)	108-67-8	-	-	-	D '87	10	-	-	-	-	-	D
Trinitroglycerol	55-63-0	-	-	-	F '87	0.005	0.005	-	-	0.005	0.2	-
Trinitrotoluene (2,4,6-)	118-96-7	-	-	-	F '89	0.02	0.02	0.0005	0.02	0.002	0.1	C
Vinyl chloride	75-01-4	F	zero	0.002	F '87	3	3	0.003	0.1	-	0.002	H
Xylenes	1330-20-7	F	10	10	D '93	40	40	0.2	7	-	-	I

¹Under review.

²1998 Final Rule for Disinfectants and Disinfection By-products: The total for five haloacetic acids is 0.06 mg/L.

continuing

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories					Cancer Descriptor	
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		10-kg Child		RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)		mg/L at 10 ⁻⁴ Cancer Risk
						One-day (mg/L)	Ten-day (mg/L)					
INORGANICS												
Ammonia	7664-41-7	-	-	-	D '92	-	-	-	-	30	-	D
Antimony	7440-36-0	F	0.006	0.006	F '92	0.01	0.01	0.0004	0.01	0.006	-	D
Arsenic	7440-38-2	F	zero	0.01	-	-	-	0.0003	0.01	-	0.002	A
Asbestos (fibers/l >10Fm length)	1332-21-4	F	7 MFL ¹	7 MFL	-	-	-	-	-	-	700-MFL	A ²
Barium	7440-39-3	F	2	2	D '93	0.7	0.7	0.2	7	-	-	N
Beryllium	7440-41-7	F	0.004	0.004	F '92	30	30	0.002	0.07	-	-	-
Boron	7440-42-8	-	-	-	F '08	3	3	0.2	7	6	-	I
Bromate	7789-38-0	F	zero	0.01	D '98	0.2	-	0.004	0.14	-	0.005	B2
Cadmium	7440-43-9	F	0.005	0.005	F '87	0.04	0.04	0.0005	0.02	0.005	-	D
Chloramine ³	10599-90-3	F	4 ⁴	4 ⁴	D '95	-	-	0.1	3.5	3.0	-	-
Chlorine	7782-50-5	F	4 ⁴	4 ⁴	D '95	3	3	0.1	5	4	-	D
Chlorine dioxide	10049-04-4	F	0.8 ⁴	0.8 ⁴	D '98	0.8	0.8	0.03	1	0.8	-	D
Chlorite	7758-19-2	F	0.8	1	D '98	0.8	0.8	0.03	1	0.8	-	D
Chromium (total)	7440-47-3	F	0.1	0.1	F '87	1	1	0.003⁵	0.1	-	-	D
Copper (at tap)	7440-50-8	F	1.3	TT ⁶	D '98	-	-	-	-	-	-	D
Cyanide	143-33-9	F	0.2	0.2	F '87	0.2	0.2	0.0006⁷	-	-	-	I
Fluoride	7681-49-4	F	4	4	-	- ⁸	-	0.06⁹	-	-	-	-
Lead (at tap)	7439-92-1	F	zero	TT ⁶	-	-	-	-	-	-	-	B2
Manganese	7439-96-5	-	-	-	F '04	1	1	0.14 ¹⁰	1.6	0.3	-	D
Mercury (inorganic)	7487-94-7	F	0.002	0.002	F '87	0.002	0.002	0.0003	0.01	0.002	-	D
Molybdenum	7439-98-7	-	-	-	D '93	0.08	0.08	0.005	0.2	0.04	-	D
Nickel	7440-02-0	F	-	-	F '95	1	1	0.02	0.7	0.1	-	-

¹MFL = million fibers per liter.

²Carcinogenicity based on inhalation exposure.

³Monochloramine; measured as free chlorine.

⁴1998 Final Rule for Disinfectants and Disinfection By-products: MRDLG=Maximum Residual Disinfection Level Goal; and MRDL=Maximum Residual Disinfection Level.

⁵IRIS value for chromium VI.

⁶Copper action level 1.3 mg/L; lead action level 0.015 mg/L.

⁷This RfD is for hydrogen cyanide.

⁸In case of overfeed of the fluoridation chemical see CDC Guidelines in Engineering and Administrative Recommendations on Water Fluoridation www.cdc.gov/mmwr/preview/mmwrhtml/00039178.htm. Elevated F levels ≥ 10mg/L require action by the water system operator.

⁹Based on dental fluorosis in children, a cosmetic effect. MCLG based on skeletal fluorosis. 10 Dietary manganese. The lifetime health advisory includes a 3 fold modifying factor to account for increased bioavailability from drinking water.

continuing

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories						Cancer Descriptor
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		10-kg Child		RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10 ⁻⁴ Cancer Risk	
						One-day (mg/L)	Ten-day (mg/L)					
Nitrate (as N)	14797-55-8	F	10	10	D '93	10 ¹	10 ¹	1.6	-	-	-	-
Nitrite (as N)	14797-65-0	F	1	1	D '93	1 ¹	1 ¹	0.16	-	-	-	-
Nitrate + Nitrite (both as N)		F	10	10	D '93	-	-	-	-	-	-	-
Perchlorate ²	14797-73-0	-	-	-	I '08	-	-	0.007	0.025	0.015	-	L/N
Selenium	7782-49-2	F	0.05	0.05	-	-	-	0.005	0.2	0.05	-	D
Silver	7440-22-4	-	0.1	0.1	F '92	0.2	0.2	0.005 ³	0.2	0.1 ³	-	D
Strontium	7440-24-6	-	-	-	D '93	25	25	0.6	20	4	-	D
Thallium	7440-28-0	F	0.0005	0.002	F '92	0.007	0.007	-	-	-	-	I
White phosphorous	7723-14-0	-	-	-	F '90	-	-	0.00002	0.0005	0.0001	-	D
Zinc	7440-66-6	-	-	-	D '93	6	6	0.3	10	2	-	I
RADIONUCLIDES												
Beta particle and photon activity (formerly man-made radionuclides)		F	zero	4 mrem/yr	-	-	-	-	-	-	4 mrem/yr	A
Gross alpha particle activity		F	zero	15 pCi/L	-	-	-	-	-	-	15 pCi/L	A
Combined Radium 226 & 228	7440-14-4	F	zero	5 pCi/L	-	-	-	-	-	-	-	A
Radon	10043-92-2	P	zero	300 pCi/L AMCL ⁴ 4000 pCi/L	-	-	-	-	-	-	150 pCi/L	A
Uranium	7440-61-1	F	zero	0.03	-	-	-	0.0006 ⁵	0.02	-	-	A

¹These values are calculated for a 4-kg infant and are protective for all age groups.

²Subchronic value for pregnant women.

³Based on a cosmetic effect.

⁴AMCL = Alternative Maximum Contaminant Level.

⁵Soluble uranium salts. Radionuclide Rule.

Table A.3: Microbiology

	Status Reg 1.	Status HA Document	MCLG	MCIL	Water Treatment Rule 111.1e
<i>Cryptosporidium</i>	F	F 01	zero	TT	Systems that enter must remove 99% of <i>Cryptosporidium</i>
<i>Cyprinidrospermosin</i>	-	F 15	-	-	-
<i>Cyanobacterial Microcystin Toxins</i>	-	F 15	-	-	-
<i>Giardia lamblia</i>	F	F 98	zero	TT	99_9% killed/inactivated
<i>Legionella</i>	f1	F 01	zero	TT	No limit; EPA believes that if <i>Giardia</i> and virus, es are inactivated, <i>Legionella</i> will also be controlled
Heterotrophic Plate Count (HPC)	f1	-	INA	TT	No more than 500 bacterial colonies per milliliter
Mycobacteria	-	F 99	-	-	-
Total Coliforms	F	-	zero	5%	No more than 5% samples total coliform positive in a month. Every sample that has total coliforms must be analyzed for fecal coliforms; no fecal coliforms are allowed.
Turbidity	F	-	INA	TT	At no time can turbidity go above 5 NTU (nephelometric turbidity units)
Viruses	f1	-	zero	TT	99_99% killed/inactivated

الخلاصة

يعد تلوث المياه بالعوامل الفيزيائية والكيميائية والبيولوجية ظاهرة حتمية تتطلب تقنية معينة للسيطرة على هذا التلوث. ان مياه الشرب التي تمر عبر شبكة توزيع المياه قد تكون معرضة لخطر التلوث بسبب تصدع الأنابيب أو عمليات إصلاحها أو توصيلها أو البناء. في هذه الدراسة، تم استخدام طريقة حديثة، وهي طلاء أنابيب مياه الشرب البلدية جزئياً بجسيمات الفضة النانوية (AgNPs). تم تحضير جسيمات الفضة النانوية بطريقة التوليف الأخضر من المستخلص المائي لأوراق نبات (*Azadirachta indica*)، تم تشخيص جسيمات الفضة النانوية بواسطة الطيف المرئي فوق البنفسجي (UV-Vis)، طيف فورييه للأشعة تحت الحمراء (FTIR)، حيود الأشعة السينية (XRD)، الانبعاث الميداني - المسح المجهر الإلكتروني (FE-SEM) والمجهر الإلكتروني للإرسال (TEM). تم تقسيم أربعة أنابيب PVC (طول 3 م وقطر 2.54 سم) إلى أطوال 30 و 50 سم. تم طلاء نصف العدد من هذه الأنابيب بجسيمات الفضة النانوية باستخدام طريقة التلدين بتيار الهواء الساخن، وتم تشخيصها باستخدام تقنيات FE-SEM و XRD. تم تهيئة أربعة أنظمة إعداد تجريبية لمحاكاة طريقة ضخ المياه في أنظمة التوزيع التقليدية.

تم اختبار الفعالية المضادة للبكتيريا ضد الإشريكية القولونية ATCC 25922 (*E. coli*) والزانفة الزنجارية ATCC 27853 (*P. aeruginosa*)، ففي حالة اختبار الإشريكية القولونية، عند مرور الماء الملوث بالبكتيريا عبر الأنابيب بطول 30 سم، وصل معدل التثبيط إلى 100% عند تركيز 125 جزء في المليون بعد 15 دقيقة، بينما عند التركيز 250 جزء في المليون، وصل معدل التثبيط إلى 100% بعد 10 دقائق. في حالة الأنابيب بطول 50 سم فقد بلغ معدل التثبيط 100% بتركيز 125 جزء في المليون بعد 20 دقيقة، بينما عند التركيز 250 جزء في المليون وصل معدل التثبيط إلى 100% بعد 15 دقيقة.

أما بالنسبة لبكتريا الزانفة الزنجارية، فعند مرور الماء عبر الأنابيب بطول 30 سم وصل معدل التثبيط إلى 99.65% بتركيز 125 جزء في المليون بعد 20 دقيقة و 100% بتركيز 250 جزء في المليون بعد 10 دقائق. عند الأنابيب ذات طول 50 سم، وصل معدل التثبيط 98.69% عند 125 جزء في المليون بعد 20 دقيقة، بينما عند 250 جزء في المليون وصل معدل التثبيط إلى 100% بعد 15 دقيقة.

تم مطابقة نتائج محتوى الفضة (Ag) في الماء المار عبر الأنابيب المطلية باستخدام مطيافية الامتصاص الذري (AAS) لتتوافق مع معايير US-EPA ومنظمة الصحة العالمية.

تم اختبار تأثير السمية الخلوية لـ AgNPs على خلايا الدم البيضاء (WBCs)، وأظهرت النتائج التي تم الحصول عليها أنه لا توجد فروق ذات دلالة إحصائية ($P \leq 0.05$) بين المجموعة

المختبرة والمجموعة الضابطة في أوقات التعرض المختلفة مع كلا النوعين من طرق التعرض، عندما تم حساب معدل التثبيط، حيث بلغت أعلى قيمة تثبيط 2.68% عند تركيز AgNPs البالغ 250 جزء في المليون في حالة التعرض المباشر.

من نتائج اختبارات التوافق الدموي لـ AgNPs مع كرات الدم الحمراء بتركيزات مختلفة من AgNPs وأوقات مختلفة، لوحظ أنه AgNPs لم تتسبب في انحلال دم معنوي ($P \leq 0.05$) في عينات الدم، حيث كان أعلى معدل لانحلال الدم 1.78% في تركيز AgNPs البالغ 250 جزء في المليون في كلا النوعين من التعرض دون فروق ذات دلالة إحصائية.



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية الهندسة
قسم الهندسة البيئية

تقييم الفعالية الميكروبية لأنابيب مياه الشرب

البلاستيكية المطلية بالفضة النانوية

رسالة مقدمة الى كلية الهندسة جامعة بابل وهي جزء من متطلبات
نيل درجة الماجستير في الهندسة / هندسة البيئة

من قبل

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