WATER PURIFICATION AND ANTIBACTERIAL EFFECTS OF METALLIC
NANOPARTICLES DEPOSITED USING DC HIGH VACUUM MAGNETRON
SPUTTERING ON FILTERING MATERIALS

By

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WATER PURIFICATION AND ANTIBACTERIAL EFFECTS OF METALLIC NANO PARTICLES DEPOSITED USING DC HIGH VACUUM MAGNETRON SPUTTERING ON FILTERING MATERIALS

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ABSTRACT

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WATER PURIFICATION AND ANTIBACTERIAL EFFECTS OF METALLIC NANOPARTICLES DEPOSITED USING DC HIGH VACUUM MAGNETRON SPUTTERING ON FILTERING MATERIALS

Under the direction of DORINA MIHUT, PH.D

The research is testing the antibacterial effects of Silver, Copper, Titanium, Zirconium and Aluminum metallic nanoparticles deposited on microsize filtration materials. The DC High Vacuum Magnetron Sputtering Equipment was used for the deposition of metallic nanoparticles. The thickness of the coatings was in-situ monitored using quartz crystal microbalance and ex-situ evaluated using a profilometer. The chemical composition of the structures was characterized using the X-Ray diffraction analysis and their surface morphology was investigated using digital optical microscopy and scanning electron microscopy. Each metallic material was deposited on filter paper with different thicknesses. The antibacterial effect was tested according to standardized methods for the examination of water and wastewater. The testing media containing the bacterial samples was contaminated water collected from the wastewater basins. The
water was initially tested for the bacterial content as collected and then exposed to metallic deposited filtering materials; the remaining targeted bacteria was quantified. The antibacterial effects of metallic nanoparticles were observed and analyzed. Deactivation rates for fecal coliform and Escherichia coli were measured for different metals with varying metallic thickness coatings. All metallic nanoparticles showed a good adhesion at microscopic level to water filter paper as observed by digital microscopy and scanning electron microscopy examination. Titanium nanoparticles did not have antibacterial effect showing no change in time evolution of E. Coli and Total Coliforms for the control and titanium coated samples. Zirconium and Aluminum nanoparticles had some antibacterial effect showing a small change in time evolution of E. Coli and Total Coliforms for the control and coated samples. It was observed that over time, silver and copper nanoparticles coated filters gradually removed both E. Coli and Total Coliforms. Additionally, this research is investigating the synergistic antibacterial effect obtained by using metallic thin films deposited on water filter paper and the effect of the potential applied to the electrically conductive structures. Silver and copper thin films (different thicknesses) deposited on water filter papers have been studied.
CHAPTER 1

INTRODUCTION AND RELATED LITERATURE

Current Situation And Problems

Providing clean drinking water to everyone is a difficult task, especially in developing countries where people may have to drink unclean or non-purified water which can lead to many diseases and potential death. Diseases are caused by bacteria and other pathogens in unclean drinking water. Some of possible microorganisms are: *Cryptosporidium*, *E. Coli*, *Hepatitis A*, and *Giardia intestinalis*. Water treatment methods have been implemented and improved over two centuries. In 1864, the use of chlorine disinfection by John Snow marked the end of common waterborne illnesses as cholera and typhoid. However, other diseases from unclean drinking water are still the top cause for death in many developing nations. According to the 2015 World Health Organization (reference WHO) report, about 2.1 billion people still lack access to safe water [1]. In addition, WHO reported that about near 1 million people are estimated to die each year from bacteria induced diarrhea because of unsafe drinking-water, poor sanitation, and lack of hygiene [1]. Moreover, few studies showed that chemical disinfectants commonly used in water cleaning industry including free chlorine, chloramines and ozone can react with various constituents in natural water and form harmful disinfection byproducts. Antimicrobial nanomaterials for water disinfection. Disinfection byproducts have been studied and it has been found out that they can have negative effects on human body. For
example, the process of water treatment using chlorine disinfection can form organic compounds called trihalomethane formation which were linked to cancer [1,2,3].

Different types of bacteria are potentially life-threatening agents, as they are able to spread dangerous diseases. One of the most famous bacteria was *Yersinia pestis* which was the cause of the “Black Death” during the 14th century. This plague was one of the most devastating disease outbreaks in human history because it killed 50 million people worldwide [4].

In the beginning of the 20th century, antibiotics were developed to provide temporary relief to infectious bacteria. Since then, many new types of antibiotics have been developed and explored during the following decades that improved significantly the human life. However, multiple studies showed that different bacteria evolved, found ways to fight against antibiotics. Overtime, this bacteria evolution provided some types of “multi drug resistance” bacteria. Antibiotics development did not produce recently effective solutions against the rapidly increasing number of resistant bacterial strains [5].

Therefore, researchers are working toward finding new promising tools against bacteria resistant strains possibly by replacing the traditional antibiotic drugs. One of the most promising solution against bacteria is using nanoparticles. Many studies showed that specific nanoparticles have toxic effects against several bacterial strains during in vitro studies [4, 5, 6, 7, 8]. Because of these results, nanoparticles could have promising effects for several applications such as: biomedical, tissue engineering, and water treatment.
Since early of the 21st century, nanomaterials have been widely studied for their antibacterial effect. Among a variety of nanomaterials, metallic and metallic oxides nanomaterials have been studied to show that they do have significant levels of antibacterial activity [4, 6].

Research For New Solutions

Silver Nanoparticles

There are many metallic nanoparticles that have antibacterial activity. Among those, silver and copper nanoparticles were studied the most due to their high effectiveness in killing bacteria. Many studies showed that silver nanoparticles can kill many types of bacteria, especially multi-drug resistance ones such as: E. Coli, Vibrio cholerae, Staphylococcus aureus, and Pseudomonas aeruginosa. In addition, many studies also suggest that the smaller size of silver nanoparticles, the more effective their antibacterial activity is [7]. A study showed that silver nanoparticles with size ranging from 1 nm to 100 nm can have a significant antibacterial effect [8]. Some studies showed that silver nanoparticles can have up to 4 different mechanisms for destroying bacteria including: DNA damage, electron transport interruption, reactive oxidation species, and protein synthesis inhibition [7, 9]. A study also gets promising results about the effectiveness of silver nanoparticles against E. Coli, V. cholerae, two main bacteria found in water that can cause severe diarrhea. This study also found out that over a period of long time, the colonies form units (CFU) for those bacteria decrease significantly after letting them in contact with silver nanoparticles. [10]. Because of its high toxicity against bacteria, silver nanoparticles have been tested widely so they can be used in diverse medical applications ranging from silver-based dressings, silver coated medicinal
devices, nanogels, and nana lotions [11]. Water treatment is another important application that may use silver nanoparticles in the future. A journal article showed that silver nanoparticles can be coated on polyurethane foam, which can work as a potential water filter. Additionally, the study also founded that silver nanoparticles attached very well to the foam and were not washed away by water flows [12]. Another study showed that silver nanoparticles can be used as bactericidal agent for water disinfection [13]. However, a study suggest that silver ions or nanoparticles could cause adverse effect on humans as well as the environment [14].

Even though silver nanoparticles have high antibacterial effect, they are costly to be produced, especially in nano-size. In addition, there are studies and reports that suggest that silver nanoparticles can cause adverse effects on both humans and the environment [15].

Reports are showing that a great amount of silver is released into the environment from industrial wastes [15, 16, 17] and that silver ions can be formed in the aqueous phase. Free silver ions can have many toxic effects on humans such as: damage to liver and kidney, irritations to unusual changes to eye, skin, respiratory, digesting system; and changes in blood cells [15]. In addition, there have been reports indicating that silver nanoparticles cannot either discriminate between different strains of bacteria or between bacteria and living cells [16,17]. Therefore, they can destroy friendly bacteria that are beneficial to the ecology, especially in the soil [16]. Silver nanoparticles could have toxic effects against bacteria that contribute to the denitrification process which is essential for the plants [16, 17]. Loss of environmental denitrification through reduction of plant productivity can lead to ecosystem problems for of rivers, lakes, and marine [17]. Hence,
it is important that more studies should be carried out to assess the toxicity effect silver nanoparticles before a conclusion on its toxicity is reached.

Copper Nanoparticles

In addition, medical studies showed that copper is required for some physiological functions of human body. Compared to silver nanoparticles, many studies showed that copper nanoparticles have similarly high level of antibacterial effect while they cost less and may be allowed to present in human body.

Therefore, many recent studies focus on copper nanoparticles as cost-effective alternative to silver nanoparticles [18, 19, 20]. A study showed that copper nanoparticles can destroy many types of both bacteria and fungus. The bacteria tested in the study were: *Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*, and *P. aeruginosa*, while the fungus tested are: *Aspergillus flavus, Aspergillus niger* and *Candida albicans* [18]. A study showed that copper nanoparticles can kill a significant amount of *E. Coli* bacteria. The study also successfully showed that copper nanoparticles can be well attached to water filter paper fiber [19]. Last but not least, a study showed that the combination of silver and copper nanoparticles have a very high antibacterial rate, can be up to 99% due to more mechanisms in destroying bacteria than silver or copper nanoparticles alone [20].
Metallic Oxides Nanoparticles

Not only metallic nanoparticles can have high antibacterial effect, but also some metallic oxides can have this property such as: zinc oxide, alumina, titanium dioxide, magnesium oxide, and calcium oxide nanoparticles [7, 9, 21]. A scientific review for established studies showed that zinc oxide nanoparticles have high antibacterial effect if their size ranges from 20 nm to 40 nm. Below 20 nm, zinc oxide nanoparticles have minimal antibacterial effect while above 40 nm, their antibacterial effect does not increase with the increase of the nanoparticle size [9]. In addition, the review suggests that titanium dioxide nanoparticles do not have antibacterial effect in dark room conditions. They only have antibacterial activity under photoactivation and for nanoparticle sizes between 40 nm and 60 nm. For alumina nanoparticles, established studies showed that the effective range of the nanoparticle size is from 50 nm to 70 nm [9]. Among metallic oxides founded to have antibacterial effect, zinc oxide nanoparticles so far can kill the greatest number of microorganisms such as: *K. pneumonias*, *P. aeruginosa*, *A. baumannii, E. Coli, Proteus mirabilis, Staphylococcus epidermis, Streptococcus pneumoniae, Salmonella typhi, Bacillus subtilis, S. aureus, and Micrococcus luteus* [21]. Lastly, magnesium oxide, and calcium oxide nanoparticles are reported to have the ability to cause damage to bacteria’s cell membrane, so they are effective against gram positive and negative bacteria such as *E. Coli* and *S. aureus* [7]. Compared to most effective metallic nanoparticles, metallic oxides are usually required to have bigger nanoparticle size to have for similar antibacterial activity [7, 9, 21].
Metal Position In The Periodic Table And Correlation With Antibacterial Effectiveness

A study reported that the position of the elements in the groups of the Periodic table may determine the effectiveness of metallic particles antibacterial effect [22]. This study investigated the antibacterial properties of silver, copper, aluminum, zinc, brass, bronze, tin and silicon against *E. Coli* in contaminated water. For performing this study different metallic thin plates, were immersed in contaminated water samples at various time intervals at room temperature. The study reported the results of experiments in the form of the number of Total coliform and *E. Coli* (CFU/ml) vs time. The results show that copper, silver and zinc have the highest rate of destroying the bacteria, followed by aluminum, and the least effective metals were tin and silicon. The author of study thought that the antibacterial activity of metal particles may depend on the group they belong to in the periodic table. Results in this study suggested that the higher the group the lower their efficacy. Silver and copper are in group I B, Zinc is in group II B, Aluminum is in group III A, while tin and silicon are in group IV A. Moreover, the study found that the more the exposure surface area of the metals, the faster they were able to destroy the bacteria. [22]

This study and other research journals have many commons about antibacterial effectiveness of metals and metal oxides nanoparticles. Many studies showed that silver and copper are the most effective antibacterial materials and they belong to group I B in the Periodic table [7, 22]. Gold nanoparticles also belongs to group I B is also reported to have significant mechanism to destroy bacteria as well [23], however they are very expensive. In addition, oxides such as: ZnO, MgO and CaO (Zn is in group II B, Mg and Ca belongs to group II A) have been studied to have promising antibacterial effects [7,
21, 22]. Alumina nanoparticles (Al is in group III A) was also found out to have antibacterial activity, however not as effective as Ag or Cu nanoparticles [7,21, 22]. Lastly, titanium dioxide and zirconium dioxide (Ti and Zr are in group IV B) were reported to have promising antibacterial effects as well [7, 21, 22]. Overall, metal and metal oxide nanoparticles are found to have antibacterial effects when the elements belong to group I A, IB, IIA, IIB, and III A of the Periodic table [7, 21, 22,23].

Mechanism Of Killing Bacteria For Metallic Nanoparticles

Metallic nanoparticles can share some similar mechanisms, but also some different ones for killing bacteria. A review study shows that silver nanoparticles can have up to four mechanisms of killing bacteria including: DNA damage, interrupt electron transport, ROS generation and induction of oxidative stress, and inhibit protein synthesis [7]. The study also shows that gold nanoparticles have only one mechanism that is the inhibition of protein synthesis [7]. Many studies [7, 21, 22] find out that most metallic nanoparticles such as silver, copper and gold have the ability of causing membrane damage to harmful bacteria. Another review study [8] shows that the membrane disruption mechanism comes from free radicals produced during the interaction between nanomaterial and the cells. These radicals may cause membrane damage, cause DNA destruction, and hinder protein function [8]. In general, most metallic nanoparticles shared one effective killing mechanism which is the cell membrane damage.

Presented in Figure 1 is the schematic representation of various antibacterial mechanisms for different nanoparticles against multi-drugs-resistant bacteria. [7]
Figure 1. Schematic representation of various antibacterial mechanisms for different nanoparticles against Multi-Drug-Resistant bacteria [7].

Cell Membrane Damage Mechanism

A study reported that silver nanoparticles can anchor to the cell wall of bacteria and penetrate it, leading to cause structural changes in the cell membrane like the permeability of the cell membrane and death of the cell [8]. There is the possibility of pits formation on the cell surface, and there is the possibility of nanoparticles accumulation on the cell surface [8]. The formation of free radicals by the silver nanoparticles was considered as another mechanism by which the cells die [8]. There have been electron spin resonance spectroscopy studies that suggested that there is formation of free radicals by the silver nanoparticles when in contact with the bacteria, and these free radicals can damage the cell membrane and make it porous which can ultimately lead to cell death as shown in Figure 2 below [8].
A research [23] was able to capture images using the Transmission Electron Microscopy technique showing that gold nanoparticles can cause damage to the membrane of a bacteria cell as shown in Figure 3 below [23].
Figure 3. “Intracellular mode of action imaged by HR-TEM. (A): untreated bacterial cell. (B): AuNPs induce vacuole formation in cytoplasm. (C): AuNPs-laser induced cellular destruction.” [23]

Reactive Oxygen Species (ROS) And DNA Damage Mechanism

It has also been proposed that there can be release of silver ions by the nanoparticles [13], and these ions can interact with the essential enzymes of bacteria and inactivate them [21]. The bacterial cells in contact with silver take in silver ions, which inhibit several functions in the cell and damage the cells. One significant inhibition is reactive oxygen species, which are produced possibly through the inhibition of a respiratory enzyme by silver ions and attack the cell itself. Silver is an acid, there is a natural tendency of an acid to react with a base [6]. The cells are majorly made up of sulfur and phosphorus which are bases. The action of these nanoparticles on the cell can cause the reaction to take place and subsequently lead to cell death. Another fact is that the DNA has sulfur and phosphorus as its major components; the nanoparticles can act on these soft bases and destroy the DNA which would lead to cell death [6]. The interaction
of the silver nanoparticles with the sulfur and phosphorus of the DNA can lead to problems in the DNA replication of the bacteria and thus terminate the microbes [6].

Objective Of The Research

The aim of this research is to observe the benefits of coated metallic nanoparticles for water disinfection by eliminating of a known type of dangerous bacteria strain that can be found in waste water. This research is based on observing *E. Coli* and other coliform bacteria as common type of bacteria that live both in human and animal intestines and are found in large quantities in waste water. The presence of *E. Coli* is a health hazard and can induce illness or death in humans, therefore it is becoming important to find methods to eliminate it from water [22,24]. Different chemical and physical based processes are available to produce metallic and oxides nanoparticles; however chemical processes could leave behind toxic chemicals that preclude their use for medical or environmental related applications [8, 10]. DC high vacuum magnetron sputtering physical vapor deposition is an environmentally friendly method that can have low substrate temperatures during metallic deposition, close to room temperature, while it is also can show good adhesion of the metallic nanoparticles to the substrate. This method was selected for the current research to coat silver, copper, titanium, zirconium, aluminum, and combination of silver and copper nanoparticles onto the commercially available filtration materials. This goal is to observe their antibacterial activity before and after the exposure to *Escherichia coli* and other coliforms from contaminated water. There are studies showing that coating thickness is an important parameter of antibacterial activity. However, thickness does reach a plateau after which further thickness increase does not improve the antibacterial activity anymore.
CHAPTER 2: EXPERIMENTAL METHODS AND EQUIPMENT

Physical Vapor Deposition (PVD) Systems

Physical Vapor Deposition (PVD) Coatings was the main method used to produce materials for the current research. During the PVD solid metals are vaporized or pulverized in a high vacuum environment ($10^{-5}$ to $10^{-8}$ torr) and deposited on substrate materials (polymers, ceramics or metals) as a pure metal or alloy coating. The material to be coated is secured in a fixture and placed in the vacuum deposition chamber. The equipment is pumped down to the optimum (base) pressure depending upon the coating materials, substrate and processes used, and the object to be coated could be preheated and sputter cleaned [26].

PVD includes evaporation and sputtering, two important methods for depositing thin films. The objective of these deposition processes is to controllably transfer atoms from a source to a substrate. During evaporation, atoms are removed from the source by thermal means, by heating the source to the gaseous stage. For sputtering, atoms are dislodged from the solid target (source) surfaces through impact with heavy gaseous ions [27].
Benefits Of Using PVD Coating

PVD could be described as a process that transfers the coating material that can be a single atom or cluster of atoms or molecules and it can provide extremely pure coatings for many applications. In addition, PVD coating processes are environmentally friendly processes that can reduce a great amount of toxic substances that must be disposed of during more conventional types of coating that involve fluid precursors and chemical reactions. For example, for the production of microchip and semiconductor device the PVD coatings enable the solar panel industry to make greener electricity as well as surgical and medical implants that require the highest degrees of purity. For aerospace and automotive industry PVD Coatings could reduce the friction coefficient for a high performance moving. For cutting tools, it produces coatings with long lasting durability due to reduced wearing which is a crucial factor. Also, PVD coatings are offering high resistant substrates to tarnishing and corrosion enabling them to be used for a wide range of decorative finishes with colors that do not fade with time. For example, the PVD coatings could be applied to watches and make them highly resistant to scratches and scrapes. They are used in a wide variety of optical applications such as glasses and self-cleaning tinted windows [26]. Their resistance to corrosion makes them widely used on household items such as door handles and plumbing fixtures. In general, PVD produces coatings with superior hardness, durability and resistance to wear, and these benefits have been applied widely in many mechanical engineering related applications [26].
High Vacuum DC Magnetron Sputtering

Vacuum Chamber

A high vacuum environment is necessary for the High DC Magnetron Sputtering process because the PVD requires a very large free path. This free path can be only achieved when the pressure of a finite volume is very low. At normal to high pressures, molecules from air can interfere with the intermolecular collisions which will reduce significantly not the purity, but the rate of the deposition of the target material (unit is Angstrom/second). In order to achieve a low-pressure/ high vacuum environment, the roughing and turbomolecular pumps are connected to the deposition chamber and are used to eliminate their molecules out of the chamber, thus reduce the pressure of the chamber over time [27].

Dry Pump

The roughing pump is used to ensure $1.0 \times 10^{-2}$ torr pressure in the chamber and works as a backing pump for the high vacuum pump. The specific pump for the research is Edwards nXDS Series Dry Scroll Pump (represented in figure 4). The Scroll Pump has two main components: a fixed scroll and a rotational scroll. The rotational scroll moves around the fixed one at a high speed to create a low to high pressure flow for air molecules [27, 28].
The Kurt J Lesker Company uses the nEXT85D pump (represented in Figure 5) to achieve high to ultra-high vacuum environment. The Turbomolecular pump uses a series of fan-like turbines to direct gas out of a chamber. The pumping principle is that the gas molecules are impelled by the rotor (the spinning blades) into the stator (stationary) blades towards higher concentrations of gas. A lower pressure environment is achieved by directing the gas molecules from the low-pressure inlet to the higher-pressure exhaust port. The high vacuum that can be achieved in the deposition chamber by using the turbomolecular pump is in the range of $8.0 \times 10^{-6}$ Torr. [27, 29]
Glow Discharges, Plasma And Collision During The Deposition Process

The word plasma was first attributed to Irving Langmuir and it is described as a quasimetric gas that behaves in a collective behavior in the presence of an applied electromagnetic field [27]. The target contains the materials to be deposited and is connected to the negative terminal of a DC power supply (cathode) and the substrate is the anode (the positive terminal of a DC power supply). The High DC Magnetron sputtering process requires a range of values for voltage between 0.3 to 0.6 kV [30, 31]. After achieving high vacuum (base pressure about \(10^{-6}\) Torr), argon gas is introduced in the chamber using the MKS 946 Vacuum System Controller and serves as the medium in which the discharge is initiated and sustained [27, 30]. Gas pressures after the argon is introduced in the chamber is in the range of \(2 \times 10^{-3}\) torr. After a visible glow discharge is maintained between the electrodes the argon positive ions in the discharge strike the cathode plate and eject the neutral target atoms through momentum transfer.
These atoms will have a relatively free traveling path towards the substrate due to the vacuum initially created in the chamber. They enter and pass through the discharge region to eventually deposit on the substrate producing a growing film. Figure 6 below represents the argon ionization during the deposition process.

![Diagram of argon ionization during deposition process](image)

**Figure 6.** Argon Ionization of High DC Magnetron. [53]

There are some steps for the process of glow discharge to occur. First, because of the small number of initial charge carriers in the system, there is only a very small current flow. More energy is introduced to the charged particles to create more carriers when the voltage increases. This occurs due to ion collisions with the cathode, which release secondary electrons, and impact ionization of neutral gas atoms. Then, the current
increases while the voltage remains constant. This regime is called the Townsend discharge which means large numbers of electrons and ions are created through avalanches. The discharge is becoming self-sustained, and the gas begins to glow. Initially, ion bombardment of the cathode is concentrated near the cathode edges or at other surface irregularities. When more power (increasing voltage) is applied, the bombardment increasingly spreads over the entire surface. The regime is now called abnormal discharge and it is the operative domain for sputtering and other discharge processes [27, 31, 32].

Unbalanced Magnetron System

For a magnetron sputtering deposition, an unbalanced magnetron is used because of stronger magnets on the outside resulting in the expansion of the plasma away from the surface of the target towards the substrate. The unbalanced magnetic field effectively trap fast moving secondary electrons that escape from the target surface. These electrons undergo ionizing collisions with neutral gas atoms like Argon and produce a greater number of both ions and electrons in the chamber [33].

![Diagram of Unbalanced Magnetic Field](image33.png)

*Figure 7. Unbalanced magnetic field [33]*
Figure 7 represents an unbalanced magnetron. The outer North poles are stronger than the inner South poles, so the field lines stretch further into the vacuum chamber. The use of unbalanced magnetron is to increase the quality of the coatings significantly [33].

Pressure Measurement

Table 1 represent pressure range of some environments.

Table 1. Pressure Level Ranges [34]

<table>
<thead>
<tr>
<th>Pressure Level</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atmospheric Pressure</td>
<td>760 Torr</td>
</tr>
<tr>
<td>Low Vacuum (Rough)</td>
<td>760 to 25 Torr</td>
</tr>
<tr>
<td>Medium Vacuum (Rough)</td>
<td>25 to 1 x 10^-3 Torr</td>
</tr>
<tr>
<td>High Vacuum</td>
<td>1 x 10^-3 to 1 x 10^-9 Torr</td>
</tr>
<tr>
<td>Ultrahigh Vacuum</td>
<td>1 x 10^-9 to 1x 10^-12 Torr</td>
</tr>
</tbody>
</table>

The pressure inside the chamber throughout the process is monitored using different pressure gauges for different pressure ranges. Among these, a hot-filament ionization pressure gauge named Bayard-Alpert is used for high vacuum (low pressures). This gauge operates by ionizing gas molecules and collecting these ions on a thin ion collector wire. This gauge can measure the pressure in the range of 5 x 10^-2 to 1 x 10^-9 Torr. A diagram of the Bayard-Alpert system is shown in figure 8. [35, 36]
Deposition Process

The roughing and turbomolecular pumps are both used to create a high vacuum environment for the deposition chamber. The substrate material to be coated is placed inside the deposition chamber on a rotating plate and the “target” material which will be applied on the substrate is placed at a certain distance from the substrate. A heavy inert gas, such as Argon, is introduced into the chamber while a direct current electric charge of a high voltage is applied to the target and substrate creating a positive and a negative electrode. The free electrons accelerate away from the negative electrode and approach the outer shell electrons of neutral gas atoms (Ar) found in their path and take away outer shell electrons of the gas atoms. Therefore, the initial neutral gas atoms become positively charged ions (Ar +). Next, the ionized argon atoms are attracted to the negative electrode and strike atoms off the target. The impact causes atoms of the target material
to become dislodged, and travel to the substrate material. The dislodged atoms are traveling on a free path (due to high vacuum) towards the substrate material and create a highly uniform layer of coating. Additionally, during the process, more electrons are produced and free electrons also travel back to the outer electron shells of the ions, so these ions change back to neutral gas atoms. The outer shell electrons return to a ground state, so the resultant neutral atom gas gains energy. Therefore, they must lose that same energy in the form of a photon, according to the conservation of energy. The release of these photons creates a glowing plasma during the sputtering process. [27, 30, 31].

*Figure 9* represents the physical view and *figure 10* represents the schematic view of the deposition system used for the research.[30, 31]
Coating Thickness Determination

Inside the vacuum chamber, there is usually a difference in distance between the sensor and substrate which will cause an incorrect reading of the actual thickness of the coating. In other words, the value displayed on the monitor of the crystal quartz balance is not accurate when using default settings of the coating machine. A mathematical value named “tooling factor” needs to be established. The coating thickness monitored in - situ using a quartz crystal microbalance and ex-situ measurements were made with a KLA Tencor Profilometer. Prior experiments were performed in order to establish the thickness versus time correspondence for accurate deposition of the desired amount of metallic nanoparticles. The substrate was continuously rotated during deposition to ensure the coating uniformity. Both sides of the filter paper were coated with metallic nanoparticles, using similar parameters.
Crystal Quartz Balance

The crystal quartz balance for the High DC magnetron sputtering measures a mass variation per unit area by measuring the change in frequency of a crystal quartz resonator. When the deposition occurs, the oscillation frequency will drop as the crystal’s mass is increased by the material (target) deposited on it. An electronic instrument continuously reads and converts frequency data to thickness data and display the values of coating thickness on the monitor [37]. *Figure 11* represent the crystal quartz balance and the monitor of the magnetron deposition system [37, 38].

*Figure 11.* Crystal quartz Balance and its monitor [37, 38]
Tooling Factor

It is a correction for the difference in material deposited on the quartz sensor versus the substrate. The value of tooling factor can be bigger or smaller than 100 depending on the location of the substrate, as shown in figure 12 below. The bigger values for tooling factor also mean higher deposition rate of the target material while the smaller values have the opposite meaning [39].

Figure 12. Position of Crystal Quart Balance affecting Tooling Factor [39]

Profile Measurements/ Profilometry

Profilometry is a technique used to extract topographical data from a surface of an experimental sample. The goals of profilometry are to get surface morphology, step heights and surface roughness. Profilometers can use a physical probe or use light. All profilometers consist of two parts – a detector and a stage. The detector determines the
locations of the points on the sample and the stage holds the experimental sample. A stylus profilometer uses a moving probe to acquire the surface height and the surface roughness. When the probe scans along the surface of the sample, there is a feedback loop that monitors the force from the sample pushing up against the probe [40]. Figure 13 represents the KLA Tencor Profilometer using for the coating thickness determination [41].

![Figure 13. KLA Tencor Profilometer [41]](image)

Procedure To Deposit Accurate Desired Amount Of Coating On The Substrate

The Tooling factor of the Kurt J. Lesker Nano 36 coating machine is set to 100 which is called “Tooling Approximate”. Values for density and Z-Factor of the target material are inputted. Experimentally, about 1-micron thickness of the coating is
deposited on the substrate which has a flat surface sample. Also, the value of the thickness on the monitor of the coating machine will be recorded as the “ThicknessQCM.”. A profilometer is used to measure the actual film thickness of the sample which is labeled as “Thickness Actual”. The actual tooling factor is calculated following the equation 1 below. The new calculated “Tooling actual” is inputted as the Tooling factor for a new deposition, so that an accurate value of desired amount of material will be displayed on the monitor of the coating machine.

Equation 1: Tooling Factor’s formula [30]:

\[
\text{Tooling}_{\text{Actual}} = \text{Tooling}_{\text{Approximate}} \times \frac{\text{Thickness}_{\text{Actual}}}{\text{Thickness}_{\text{QCM}}}
\]

In sum, prior experiments were performed in order to establish the thickness versus time correspondence for accurate deposition of the desired amount of metallic nanoparticles. The substrate was continuously rotated during deposition in order to ensure the coating uniformity. Both sides of the filter paper were coated with metallic nanoparticles, using similar parameters.

Optical And Digital Microscope

An optical microscope has the following main components: an objective lens, ocular lens, lens tube, stage, and reflector. An experimental specimen placed on the stage is magnified through the objective lens. When the target is focused, human eyes can observe a magnified image of the specimen through the ocular lens [42]. An optical microscope was used for the research initially.
Digital microscope has a very similar working principles to optical ones, the difference is that the magnified image of the sample is usually displayed on a monitor of a computer. *Figure 14* represents the Keyence digital microscope using for the research [43].

![Keyence VHX 6000 Digital Microscope](image)

*Figure 14. Keyence VHX 6000 Digital Microscope [43]*

**Scanning Electron Microscope**

The scanning electron microscope (SEM) was invented by German scientists Max Knoll and Ernst Ruska in 1930. The equipment allowed scientists to observe an experimental object at a much higher magnification compared to optical microscope. For material science research, SEM can provide a detailed examination of the surface of a material. The scanning electron microscope operates by scanning the surface of the specimen with a focused beam of electrons. The beam of electrons will excite the atoms along the surface of the sample, causing them to emit some secondary electrons. These reflected (or back-scattered) secondary electrons are collected and then displayed at the
same scanning rate on a cathode ray tube which create a detailed image of the topography of the specimen surface. Usually, a very thin metallic surface coating is applied to a nonconductive specimen, so the surface of the sample is conductive and ready for SEM. In addition, SEM can provide magnifications up to 50,000 times and information about the chemical composition of the sample. An example of the scanning electron microscope equipment used in this research is shown in figure 15 [26, 44].

![Figure 15. Scanning Electron Microscope. Usage courtesy of Georgia Institute of Technology [45]](image)

Energy-Dispersive X-Ray Spectroscopy

Energy dispersive X-Ray spectroscopy (EDS) is an analytical technique for surface analysis. The technique is based on the interaction of X-ray excitation and a
sample. When an incident beam of X-rays is focused into the sample, an electron gets excited and jumps from an outer shell to an inner shell of an atom, the difference in energy is released in the form of an X-Ray. The energy of X-rays emitted can be measured by an energy dispersive spectrometer. EDS equipment has four main components including: excitation source, X-ray detector, pulse processor and analyzer. These components work together to get the sample composition calculated and presented in graphic forms by computer software [46].

**X-Ray Diffraction**

X-ray diffraction (XRD) is used to detect the atomic crystalline structure of a material. Bragg's law has been commonly used to explain the interference pattern of X-rays scattered by crystals. The XRD phenomenon happen is when atomic planes of a crystal cause an incident beam of X-rays to interfere with one another. The interference can be either constructive (in phase) or destructive (out of phase), depending on the diffraction angle of the atomic plane they interact. The XRD system then will determine the location and measure the intensity of the diffracted X-ray waves. The constructive and destructive interference of the waves will create high and low regions of intensity at different angles. The crystalline plane structure of the material will be determined by examining the location of these high intensity peaks because each material has a unique combination of intensity peaks from scientific experiments. For this research, XRD is used as the main tool to verify orientation of crystal structure or grain, so that the purity of the metallic coatings can be confirmed before antibacterial testing [44].
Standardized Environmental Method

All the coating processes and antibacterial testing procedures of the filter papers were handled with pristine nitrile gloves to limit exposure to other bacteria from the environment. The contaminated water used for antibacterial testing was collected from Water Management Plant basins, Macon, GA, and the testing was performed according to the standard methods for the examination of water and wastewater. Same amounts of contaminated water (220 ml) were poured into four different beakers (250 ml capacity each) and exposed to the same surface filter paper (pristine and metallic coated). Different size surfaces were investigated to observe their antibacterial effects: 70 and 140 cm$^2$. The same time intervals were used to expose the contaminated water to filter papers: 0, 30, 120 and 300 minutes were used. The contaminated water was examined for the bacterial content after the exposure interval following a standard protocol [49]. The exposed contaminated water was filtered using certified 0.45 microns GN-6 Metricel ® MCE membrane disc filters of 47 cm diameter size that retained the E. Coli. The total coliform bacteria were extracted using Gast vacuum equipment. Petri dishes containing 2 ml of m-ColiBlue24® broth testing medium were used to cultivate the bacteria collected on each membrane disc filters according to standard procedures and all samples were incubated for 24 hours at a constant temperature of 35 +/- 0.5 Celsius degrees. Figure 16 represents the equipment used for the standardized environmental method.
Figure 16. Equipment used for the standardized environmental method
CHAPTER 3
RESULTS AND DISCUSSION

Results of surface morphology of coatings

The micro size water filtration materials selected as substrate for coating with silver, copper and titanium metallic nanoparticles are ensuring a high surface exposure of metallic nanoparticles to the contaminated water containing submicron size bacteria. Two figures below are showing digital microscopy images of the pristine filter paper using 500 X magnification (figure 17) and respectively the 1-micron thickness silver coated filter paper pattern using 20X magnification (figure 18). Coating thickness for all metals was evaluated as a function of time by using experimental in-situ and ex-situ thickness measurements to accurately determine the time interval required for a specific thickness deposition [47, 48, 49].

Figure 17. Pristine filter paper fibers 500X magnification.
The surface morphology of the coated filter paper was investigated using digital microscopy and it was observed a good coverage of the fibers with no delamination of the metallic coatings. Metallic nanoparticles were coated onto micron size water filter materials with a fibrous structure having high surface to volume ratio. Figure 19 is representing a dual digital image of one-micron thickness silver coated filter paper (upper half) and masked pristine filter paper (lower half) using 500 X magnification. Figure 20 is representing surface morphology of one-micron thickness titanium coating on fibers using 500X magnification. Similar images were obtained for other metallic coatings all showing good adherence of metallic nanoparticles to fibers as substrates. Figure 21 is representing typical digital microscopy images of the filter paper with copper coating (500 X magnification) showing that fibers provide large exposure surface for metallic thin films to contaminated water containing bacteria [47, 48, 49].
Figure 19. Upper half: silver coated fibers, Lower half: pristine filter (1µm thickness) 500X magnification.

Figure 20. Titanium coated fibers (1µm paper thickness) 5000X magnification.
The other types of metallic thin films on water filter paper showed similar morphologies with titanium and copper coatings. Two images below present the SEM of filter paper fibers coated with 300 nm thickness of copper in cross sectional view (figure 22) and as surface view of the fibers (figure 23) [47].
Figure 23. *SEM of 300 nm copper coated filter paper showing surface view of the fibers.*

Two images below represent the SEM of filter paper fibers coated with 300 nm thickness of silver as surface view of the fibers (*figure 24*) and the silver coating on a single fiber (*figure 25*). The figures show a good coverage of the fibers’ surface with thin films, a good adhesion of metallic nanoparticles to the fibrous substrate, and no delamination of the coatings from the fibers occurred. Similar morphologies and good adhesion were observed for all other metallic thin films deposited on the fibrous filter paper substrate [47, 48, 49].
Figure 24.  Scanning Electron Microscopy of 300 nm silver coated filter paper showing.

Figure 25.  Scanning Electron Microscopy of 300 nm silver coated filter paper showing surface view of a single fiber.
Results for X-Ray Diffraction

The X-Ray Diffraction (XRD) data were collected for metallic films deposited on glass substrates simultaneously with filter paper coating. The XRD analysis indicated that coated structures are of pure crystalline copper, silver, aluminum, zirconium and titanium. The XRD graph for silver coating on glass is presented in Figure 26 where the silver reflection peaks were observed for 2θ values of 38.11; 44.3; 64.47; and 77.42 degrees corresponding to (111), (200), (220) and (311) reflection planes (JCPDS 87-0720) and the XRD graph for copper coating on glass is presented in Figure 27 where the copper reflection peaks were observed for 2θ values of 43.12; 50.26; 74.01 and 89.82 degrees corresponding to (111), (200), (220) and (311) reflection planes (JCPDS 85-1326). Similar graphs and correspondences were found for the other metallic coatings (not presented here). The EDS analysis performed on the silver coated fibers is indicating 93.63 % silver and 6.37 % carbon content (from the paper fiber). However, for the copper coated fibers the EDS analysis is indicating 93.3 % copper, 4.7 % carbon and also small amounts (2 %) of oxygen possibly due to the thin passivation layer formed on the copper surface exposed to air. The presence of oxygen detected by EDS for copper but not for silver thin films is consistent with other researchers’ findings that also observed small amounts of oxides with the formation of copper nanoparticles on fibers [47, 48, 49].
Figure 26.  X-ray Diffraction of thin films deposited on glass substrate for silver.

Figure 27.  X-ray Diffraction of thin films deposited on glass substrate for copper.
Identical surfaces of the metallic coated filter papers were exposed to same amounts of contaminated water for the established exposure times and the standard protocol was followed to monitor their antibacterial activity. Figure 28 below is showing a typical image of a plate containing *E. Coli* and Total coliforms colonies after the contaminated water was exposed to filters. The *E. Coli* and total coliforms colonies were counted for each plate to observe the effects of various metallic nanoparticles and different exposure times [47, 48, 49].

*Figure 28.* Typical plate showing *E. Coli* (blue) and Total coliforms colonies (red and blue).
Results of antibacterial activities of various metallic nanoparticles

*Figure 29* and *Figure 30* presents data (averaged) obtained after conducting tests consisting of exposure of uncoated and metallic coated filter paper to contaminated water. The line on the graph for the control sample represents the uncoated water filter paper antibacterial behavior, and the other lines are representing the antibacterial effects of 300 nm thickness metallic coated filter paper with silver, copper, aluminum, titanium and zirconium over the course of exposure. It was observed that control and titanium coated samples did not have antibacterial effect, and similar number of *E. Coli* (*Figure 29*) and total coliforms (*Figure 30*) colonies were counted after the selected exposure times. Zirconium and aluminum coated fiber papers had some antibacterial effects with aluminum acting more effective against *E. Coli*. Both silver and copper had pronounced antibacterial effect with copper being more effective against both *E. Coli* and total coliforms than silver, therefore copper with a smaller atomic size could be a good and less expensive alternative to silver to use as a disinfectant material against coliforms. The antibacterial effectiveness results for metallic thin films were consistent with the work of researchers showing that for metals, the higher is the group number the weaker is the antibacterial effect against coliforms [47, 48].
Figure 29. Antibacterial effectiveness for control and metallic coated filter papers (300 nm thickness) against *E. Coli*.

Figure 30. Antibacterial effectiveness for control and metallic coated filter papers (300 nm thickness) against Total coliforms.
Results about the effectiveness of thickness coatings of metallic nanoparticles

The influence of coating thickness on antibacterial behavior was analyzed for 30, 300 nm and 1 μm metallic thin film thickness and similar trends were observed for the same metallic layer with different thicknesses against bacteria. Selectively, figure 31 and figure 32 shows the effectiveness against *E. Coli* for silver (a) and copper (b) thin films. It was observed that all three coating thicknesses showed similar responses. For the silver coating the line for 30 nm was located between the 300 nm and 1μm lines indicating that a plateau of the antibacterial activity could be achieved with 30 nm coating thickness. Copper coatings also showed similar responses for all three coating thicknesses; however, the 1 μm thickness showed the best effectiveness against *E. Coli* with a performance closer to 300 nm thickness. Similar trends were observed against total coliforms [47, 48].

*Figure 31.* Antibacterial effectiveness against *E. Coli* for 30, 300 nm and 1μm thickness coating on water filter paper for silver.
Figure 32. Antibacterial effectiveness against E. Coli for 30, 300 nm and 1µm thickness coating on water filter paper for copper.

Figure 33 is representing the average data obtained after three tests performed during three consecutive weeks and consisting of exposure of the uncoated and coated filter paper to contaminated water. The line on the graph for the control sample represents pristine water filter paper, and the other lines on the graph are representing the effect of 300 nm thickness metallic coated titanium, silver, copper, and silver/ copper (silver one side, copper the other side). It was observed that control and titanium coated samples did not have an antibacterial effect and a similar number of E. Coli colonies were counted after different exposure times. However, silver, copper and silver/ copper combination had a pronounced antibacterial effect, with silver/copper combination having the highest antibacterial effect. A similar response is observed in Figure 34 for Total coliform colonies, where silver had a better antibacterial effect than copper initially, but copper had a better effect for longer exposure times; the best response was for silver/ copper
combination. For both situations, titanium nanoparticles coated filter papers did not have an antibacterial effect and showed a similar behavior with the control samples [48, 49].

**Figure 33.** Antibacterial activity against *E. coli* for control and metallic coated filter papers (300 nm thickness).

**Figure 34.** Antibacterial activity against Total coliforms for control and metallic coated filter papers (300 nm thickness).
Figure 35, figure 36 and figure 37 are representing the antibacterial activity against *E. coli* (Figure 35) respectively against Total coliforms (Figure 36) for silver coated filter papers (30, 300 nm and 1µm thickness). It was observed that all three coating thicknesses showed similar responses (30 nm line for example was between the 300 nm and 1µm thickness lines) indicating that possible a plateau of the antibacterial activity was reached starting with 30 nm coating thickness or below. Similar responses were obtained for the antibacterial activity of copper and silver/copper combination, while the control and titanium coated samples had a linear response showing no antibacterial effect. *Figure 37* is showing the linear antibacterial activity against *E.coli* for water filter paper coated with titanium (30, 300 nm and 1µm thickness), showing no antibacterial effect; a similar response was also observed against Total coliforms [47, 48].

Figure 35.  *E. Coli* antibacterial activity for silver coated filter papers (30, 300 nm and 1µm thickness).
Figure 36.  Total coliforms antibacterial activity for silver coated filter papers (30, 300 nm and 1µm thickness).

Figure 37.  Antibacterial activity against E.coli for titanium coated filter papers (30, 300 nm and 1µm thickness).
The antibacterial effectiveness was further investigated for 300 nm copper, 300 nm silver thin films on filter paper in comparison with control samples and with nanolayers of copper and silver thin films in the following two arrangements: (A) 5 nm copper deposited on fibers, followed by 5 nm silver coating and for (B) 5 nm silver deposited on fibers, followed by 5 nm copper coating. Figure 38 and figure 39 represent the antibacterial effectiveness against E. Coli (figure 38) and total coliforms (figure 39) for these structures. It was observed that both 5 nm combinations of copper and silver had similar effective behavior against coliforms, comparable to 300 nm copper coatings and more effective than silver coating, 300 nm thickness. Both (A) and (B) arrangements had similar responses showing that the effects from both silver and copper nanolayers were observable at this small thickness and small quantities of metallic coatings such as 5 nm layers of copper and silver deposited on the fibers were as effective as 300 nm copper thickness against coliforms. It was observed that by applying a nanolayer of silver (e.g. 5 nm) on top of the copper coating, the antibacterial effectiveness was preserved; however, it may offer positive effects on reducing the formation of copper oxides on the surface regions of the copper thin films and subsequently prevents the possible release of copper oxides particles in water during antibacterial treatment [47, 48].
Figure 38. Antibacterial effectiveness for control, Cu and Ag 300 nm thickness coating and Cu/ Ag and Ag/ Cu 5 nm thickness coating against *E. Coli*.

Figure 39. Antibacterial effectiveness for control, Cu and Ag 300 nm thickness coating and Cu/ Ag and Ag/ Cu 5 nm thickness coating against *Total coliforms*. 
Results about the potential loss of metallic nanoparticles of nanoparticles

In order to observe the changes in the effectiveness of metallic coated filter papers after exposure to water due to potential loss of metallic nanoparticles, the metallic coated samples were immersed in distilled water in the same conditions as used during testing; they were afterwards dried and later exposed to polluted water. Figure 40 and figure 41 are showing a comparison of the antibacterial effectiveness for the uncoated filter paper and for the arrangements of (A) copper 5 nm deposited on fibers, followed by 5 nm silver coating and (B) 5 nm silver deposited on fibers, followed by 5 nm copper coating as deposited (1) and after the immersion in distilled water (2). It was observed that samples that were initially exposed to distilled water (2) did not lose their effectiveness against bacteria and had a slightly better performance against both E. Coli (figure 40) and total coliforms (figure 41) than in the case of coated samples. This shows that coated samples were able to keep their antibacterial performance after the exposure to distilled water and therefore, no changes in the efficacy of coatings were observed due to the possible loss of metallic nanoparticles in the water [47, 48].
Figure 40. Antibacterial effectiveness for control, Cu/Ag and Ag/Cu 5 nm thickness before (1) and after (2) exposure to deionized water against E. Coli.

Figure 41. Antibacterial effectiveness for control, Cu/Ag and Ag/Cu 5 nm thickness before (1) and after (2) exposure to deionized water against Total coliforms.
Summary of Discussion

Metallic thin films deposited by DC magnetron sputtering system offered a good coverage of water filter papers fibers and exhibited good adhesion at microscopic level to the paper fibers as observed by digital microscopy and SEM examination. The filter fibers provide a high area of exposure to contaminated water for metallic nanoparticles resulting in an enhanced antibacterial performance. The research tested the influence of the surface exposure size of water filter papers to contaminated water and it was found that by doubling the surface of the filter paper, the antibacterial activity was accordingly faster. For a higher surface exposure, 140 cm² versus 70 cm², the rate of antibacterial activity increased in the case of silver and copper nanoparticles, indicating that higher surface exposure equates with more intense antibacterial activity [47,48].

There were no observable changes with time in evolution of *E. Coli* and total coliforms for uncoated and titanium coated filter paper samples showing no antibacterial activity for these materials. The zirconium and aluminum nanoparticles had low antibacterial performance. It was observed that over time, both silver and copper coated filter papers showed high antibacterial performance and gradually removed both *E. Coli* and total coliforms, though copper proved to be more effective than silver [47,48].

There was no noticeable difference in the antibacterial effect of all metallic thin films with 30 nm, 300 nm and 1 µm thickness showing that higher coating thickness was not more efficient. Combinations of silver and copper coatings of 5 nm thickness (each) proved to have similar antibacterial effectiveness as compared to 300 nm thickness of copper. Small quantities of copper and silver nanoparticles can be deposited on filter
paper fibers and provide good antibacterial effectiveness while offering a relatively cost-effective method in water treatment. [47, 48].
CHAPTER 4

FUTURE WORK

This research has a great amount of potential expansion and currently is investigating the synergistic antibacterial effect obtained by using silver and copper thin films deposited on water filter paper and the effect of the potential applied to the electrically conductive structures as well as the effect of ultra-sonic sounds [48,51].

*Figure 42 below represents the schematic of the ongoing experiment [52]. A container will have an amount of sufficient waste water and a sample of silver or copper coated filter paper. Conductive wires and electrical tape will be attached to the silver or copper coated filter paper. A direct current (DC) will be applied on the silver or copper coated filter paper with the thickness of 1 micron-size. The coated nanoparticles with a high conductivity will create a closed DC electrical loop. Voltage and current values of this close loop will be recorded at various time of the experiment. Identical surfaces of the metallic coated filter papers were exposed to same amounts of contaminated water for the established exposure times and the standard protocol was followed to monitor their antibacterial activity [48, 49, 50, 51].*
Figure 42. Schematic view for the experiment of the potential applied to the electrically conductive structures of metallic coatings on water filter paper.

The experiment above was performed and the data collected gave promising results. Figure 43 and figure 44 are showing a comparison of the antibacterial effectiveness for the silver coated filter paper and for the electrical power of (A) Control, (B) No power applied, (C) Low power applied-2W, (D) High power applied-15W. There were no observable changes with time in evolution of *E. Coli* and total coliforms for uncoated filter paper samples showing no antibacterial activity for these materials. The silver coated filter paper with (B) None power applied shows low antibacterial
performance in this case. The silver coated filter paper of (C) High power applied- 15W shows better performance than (D) Low power applied 2W [48].

Figure 43. The antibacterial effectiveness for E. Coli of the silver coated filter paper and various electrical power applied.
Figure 44. The antibacterial effectiveness for Total coliforms of the silver coated filter paper and various electrical power applied.
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[45] Culp, J. (2018). The study of the properties of polymeric materials both pristine and coated with metallic nano-layers with regard to impact erosion.


