The Efficacy of Silver/Copper Nanoparticles in Biofilm Control on Water Supply Pipeline Materials.

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Abstract

Biofilms are microorganisms that inhabit inside a matrix made mainly of exopolysaccharides that are secreted from the micriobual consortia. Silver and copper ions efficacies have been proven in vitro, but, Huang, et al., (2008) confirmed the problem of biofilms altering the effects of these chemicals on the bacteria. The microbial killing effects of copper are in consequence of oxidation of the sulfhydryl groups of enzymes within the bacterial cell. The oxidation of these enzymes inhibits the enzymes activity halting the respiratory function of the cell, thus killing the bacterial cell (Pyle, et al., 1992). Silver nanoparticles increase the permeability of the cell membrane leading to cell leakage. The infiltration of silver ions in the mitochondria of the cell disrupts the ATP production by affecting the respiratory function enzymes resulting in the deformation of the chain that forms ATP. In a preceding study, biofilm bacterium were exposed to silver nanoparticles for various periods in 1mg and 2mg (per 25ml) concentrations. The results displayed a natural log delay with the required bacterial count being obtained at day 7. The research herein, was aimed at investigating the reduction of the effective time, the synergetic effects of copper nanoparticles and the application of nanoparticles in flow conditions. Concentrations of 10mg, 20mg and 50mg (per 25ml) of silver and copper nanoparticles were utilized in beaker and channel flow experimental setup varying the exposure time. A combination of copper and silver nanoparticles with concentrations 5mg ,10mg and 50mg were also tested in similar conditions to investigate the synergetic effects of the two heavy metals. In this experiment the silver nitrate and copper nitrate were reduced by citric acid and sodium hydroxide was used as the analytical agent. Given the results obtained, it is seen that the chemical application with flow aids the reduction of bacteria and the optimum concentration is between 10mg and 20mg (per 25ml) and it can be concluded that there is a synergetic effect between the silver and copper nanoparticles.

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1. Introduction

Biofilms are created by biofouling, which is the attaching of bacteria, fungi, viruses and other microorganisms to the inside surfaces of pipelines in distribution networks. Biofilm growth in distribution network pipelines lowers the quality of water supplied to the consumer, both health wise and aesthetically. Waterborne disease outbreaks have and will continue to occur due to the lowering of the water quality. This becomes a major problem if the biofilms are not cleaned out effectively.

Surface roughness is increased over time due to the bacteria accumulation, thus changing the properties the pipeline was designed for. This change in roughness might cause change in the flow patterns and alter the energy requirements needed for pumping. This change in flow can cause cavitation that accelerates the deterioration of the pipes.

In addition, these biofilms harbour harmful pathogens (bacteria, viruses, fungi, algae) and other micro-hazards. An ideal growing environment for bacteria is created by the biofilms outer mucus like layer provides protection for these, protection against certain disinfectants and the mechanical action of the water flow through the pipes. This results in delivered drinking water that has both odour, an unpleasant colour. Waterborne diseases such as typhoid, cholera and meningitis are caused by some of these bacteria such as *Escherichia coli, Klesiella pneumoniae* found in biofilms.

Access to microbiologically safe drinking water is a basic need, however, biofilm removal methods that are available to control biofilm in drinking water distribution systems are either time consuming, expensive to carry out or may lead to human health risks if not properly executed. The presence of residual disinfectants such as iodine or chlorine, from the purification plant in the water is not enough to eradicate nor mitigate this issue. There are many ways that have been employed to try to clean out pipes all of which can be grouped into four categories. One of which is to do it mechanically. Mechanically cleaning entails manually scrubbing the pipes. This type of method, however, can be labour intensive, time consuming, can be expensive and has a long down time on supply. The other of these methods makes use of biological agents which is achieved by introducing biological substances into the pipe network that will destroy or eat away the biofilm, this however is dangerous as some of these biological substances may be unsafe for human consumption and will be hard to completely flush out. Another way to remove biofilms is by altering the environment inside the pipes of the system by controlling the conditions such as

PH, temperature, hydrodynamics status and residual chemical composition. If the right environment is kept this will dramatically slow down biofilm growth. Problem is it is costly and hard to maintain such conditions in the pipe systems. The last of the four cleaning methods is to employ chemicals. This needs adequate cleansing before returning to the consumer depending on which chemicals have been made use of. The chemicals employed might also adversely react with the pipe material, giving rise to a new problem.

Silver and copper ions efficacies on bacterial eradication have been proven in vitro, but Huang, et al., (2008) indicated the problem of biofilms altering the effects of these chemicals on the bacteria and suggests further study in this area. Silver nanoparticles display a wide range of biocidal activity to many microorganisms like, Fungai, algae, bacteria and viruses (Marambio-Jones & Hoek, 2010). It is because of these properties of silver nanoparticles that its application to eradicate biofilms in water supply pipelines is being investigated.

1.2 Research Scope

If a solution can be found that is easy to implement, efficient and cost effective, this could help improve one of the most basic human right, the availability of safe drinking water and address the effects the biofilms have on the pipelines. Biofilms present a wide range of water quality and operational problems. Biofilms can be responsible for loss of distribution system disinfectant residuals, increased bacterial levels, reduction of dissolved oxygen, taste and odour changes, red or black water problems due to iron or sulphate-reducing bacteria, microbial-influenced corrosion, hydraulic roughness, and reduced materials life (Characklis and Marshal, 1990). Water quality issues can have drastic effects on consumer health. Legionarries disease is a kind of pneumonia illness that can be caused by the bacterium (Legionella pneumophila) found in water supply, even hot water reticulation systems (LIN, et al., 1996). Pseudomonas aeruginosa, Stenotrophomonas maltophilla and Acinetobacter baumannii are pathogens found in water systems and cause infections (Huang, et al., 2008). These bacteria are gram negative and found in chlorinated water systems. These pathogens have been linked to some lung infections and urinary tract infections.

1.2.1 Research scope and question

The general scope overview of this research can be summarised by the points below;

- Investigation of the antibacterial and synergetic effects of copper and silver nanoparticles in biofilms.

- Concentration of nanoparticles to achieve acceptable levels of bacterial count within 24hours or less.
- Compare the results given by a beaker setup and a channel flow setup experiment.

The general objective of this research is solely to investigate if the treatment using metal nanoparticles, namely silver and copper is effective enough to warrant further research to take it into industry. The above-mentioned research boundaries are due to the limitations listed below (1.2.2 Limitations)

1.2.2 Limitations

The limitations met by the experimental setup are listed below have suggested solutions suggested/given in Chapter 5 for future scope.

- The biofilm growth time

  The pipelines that have either have operational or water quality issues due to biofilms have mature/thicker biofilms that have been grown over extended periods of time. The experiment presented herein is limited due to the level of maturity of the biofilms that are experimented on. This poses inaccuracies in the concentrations and time it might then take in an actual pipeline.
- Growth and treatment environmental conditions

  In the experiments herein the biofilms are grown in a growth tank in lab conditions. This is a different environment as compare to the underground, high-pressured, high flow conditions that would be found in an actual pipeline. This difference can have different type/composition of biofilm present in the pipeline as compared to the coupons employed herein.
- Bacterial count area percentage

  The percentage area of the coupons imaged is less than 25%. Although image location randomisation and a minimum of 10 images per coupon are used to attempt to get the best bacterial representation of the coupon surface area, the chances are high that clusters of bacteria are missed.
- Chemical adhesion to apparatus surfaces

  The nanoparticles used have been observed to adhere to the surfaces of small diameter pipes. This will affect the concentration of nanoparticles that interact with the coupons. Figure A.5 and Figure A.2 refer.

1.2.3 Methodology outline

Below is a brief summary of the methodology of the entire research. The experimental accept of the study will be explained in depth in Chapter 3.

Literature review	-	Determine the chemical components and synthesis.
	-	Investigate antibacterial effects and health implications of the chosen chemicals.
Experimental phase	-	Biofilm growth
	-	Channel and beaker treatment
	-	Imagery and count
Results	-	Data generation
	-	Data analysis

1.3 Anticipated Results

Anticipated results of the experiment:

- Reduction in bacteria count on all samples (except the controls).
- Treatment period to achieve acceptable bacterial count below 36hours.
- Flow conditions to improve treatment time.
- Synergetic effects between silver and copper nanoparticles.

1.4 Thesis Layout

The research herein is presented as follows;

Chapter 1: Introduction - outlining the problem and giving the scope and or limitations of the research.

Chapter 2: Literature review – exploration of relevant studies and experiments pertaining the research

Chapter 3: Methodology – an outline of the experimental and data analysis methodology

Chapter 4: Results and Discussion – the display and explanation of the experimental results.

Chapter 5: Concluding remarks – recommendation stemming from results and future scope suggestion

Appendix A: Tables, equations and figures pertaining to the methodology aspect of the research

Appendix B Data Tables and Sample SEM images

2. Literature Review

Herein a review of past works and literatures pertaining to the chosen chemicals are reviewed, in addition to brief insights to their health impacts.

2.1 Biofilm Background

Chlorination has been the most widely used method of water purification since its antibacterial properties were discovered. Martinez-Gutierrez, et al., (2013) defines biofilms as microorganisms that inhabit inside a matrix made mainly of exopolysaccharides that are secreted from the microbial consortia. The biofilm shielded bacteria are resistant to antibiotics biocides and disinfectants when compared to free roaming cells. Its due to this reason that normal methods of disinfectants are not effective to biofilm (Pitts, et al., 2003). The microorganisms in the biofilm benefit from the exopolymer, or otherwise known as the slime layer. This layer offers protection from the external environment Biofilms greatly increase the antibiotic resistance of microbial organisms making it substantially more difficult to control colonization (Martinez-Gutierrez, et al., 2013). Biofilms in addition protect pathogenic organisms from adverse environmental conditions. These become reservoirs for bacteria causing disease outbreaks (Martinez-Gutierrez, et al., 2013). The shear from the fluid flowing and the antibacterial agents are some of the factors that could kill, or slow down the growth of the biofilms (Martinez-Gutierrez, et al., 2013).

2.1.1 Biofilm growth conditions

Reducing the rate of biofilm removal by limiting its formation and slowing down the regrowth, makes more cost-effective sense to pipeline maintenance. Creating an environment within the pipeline that is not conducive for the biofilms limits biofilm growth. This can be achieved by altering some factors within the pipeline, such as:

2.1.1.1 Environmental Factors

Temperature within the pipeline is a major factor that is directly linked to the microbial growth rate. All the factors are affected by the temperature of the pipeline water indirectly or directly. Temperature of the water is difficult to control, and most water treatment plants do not have access to the equipment that is required to achieve this. Temperature can therefore be indirectly controlled by changing the levels of the other factors such as, the residual concentration of

disinfectant materials. Studies show that most microbial growth and activity happens at a certain optimum temperature higher than fifteen degrees Celsius. (Anon., 1992). Lag time between entry and multiplication is also greatly affected by the temperature of the water supply network, as is the growth rate. (Anon., 1992)

Rainfall is an environmental factor that increases runoff and the microbial concentration in the untreated water. Runoff increases the amount of sedimentation in water treatment plant increasing the chances of breakthrough

2.1.1.2 Sediment Accumulation

The soil fines and particles that pass through the treatment phase increase the microbial growth rate by providing high amounts of carbon which is an essential part needed for growth. The sedimentation is not detected when breakthrough occurs because of a number of factors such as, their colour. These fines may carry with them bacteria. The low flow areas and depends of the water supply network accumulate a lot of the sedimentation. These areas become high bacterial zones.

2.1.1.3 Corrosion

Corrosion influences the rate of biofilm growth in a number of different ways, such as, reducing the water flow rate close to surface. This reduction in flow sequentially reduces shearing forces, therefore diminishing the shearing force that disturbs biofilm growth. Corrosion increases the surface roughness providing pits for bacteria to hide in. Corrosion can come about by, physical, chemical or biological action.

2.1.1.4 Disinfection Residual Concentrations

Although the presence of chlorine in the pipeline as residual disinfect minimal no effect on bacterial growth, some residual disinfectant in water supplies is needed. The absence of which results in the rapid undisturbed growth of biofilms within the pipeline.

2.1.1.5 Nutrient Availability

Certain substances are required to be the source of nutrients needed for microbial energy generation and cell multiplication the main nutrients needed for growth are phosphorous, nitrogen and carbon. Carbon is both, used for cell generation and an energy source to certain microorganisms. Bacteria (heterotrophic) need these three nutrients in a ratio of; carbon 100: nitrogen 10: phosphorus 1, carbon is usually the limiting one. carbon dioxide alone can be a source of carbon for the Bacteria. Nitrogen is used by microbial organisms to create amino acids

and genetic from. Nitrogen is released when vegetation decays, the nitrogen is dissolved and carried into raw water networks. Ammonia is the main source for nitrogen that is found naturally. Phosphate are sometimes introduced into network systems to control corrosion.

2.1.1.6 Hydraulic Effects

Increasing the flow will increase shearing, transport larger amount of disinfectant and cause greater flux of nutrients to the pipe surface this is a few examples of how altering the flow of the pipe network can affect the biofilm growth conditions (Anon., 1992). Water flow can be changed by fire hydrants, water main breaks, flushing and other factors due to the type and the design of water supply network and pipe size. Water hammer due to sudden closing of pipeline system also affects biofilm accumulation mainly by affecting sedimentation and corrosion. Water hammer could also result in pipe breakages leading to an influx of contaminates.

2.1.2 Health implications of biofilm bacteria

The bacteria and other microorganisms that inhabit the biofilm can be harmful to humans if ingested. Legionaries disease is a kind of illness that can be caused by the bacterium found in water supply, even hot water reticulation, systems (LIN, et al., 1996). These bacteria are known as *Legionella pneumophila*. Hospitals have tried to eradicate these bacteria a number of ways, for example, heat flushing, ozone UV light exposure, and hyperchlorination. These anti-bacterial methods have adverse disadvantages like, heat flushing will have bacteria grow back in short period of time and hyperchlorination will increase in corrosion of the pipe material (LIN, et al., 1996). *Pseudomonas aeruginosa, Stenotrophomonas maltophilla and Acinetobacter baumannii* are pathogens found in water systems and cause infections (Huang, et al., 2008). These bacteria are gram negative and found in chlorinated water systems. These pathogens have been linked to some lung infections and urinary tract infections in hospitals.

2.1.3 Biofilm control methods

There are many ways that have been employed to try to clean out pipes all of which can be grouped into four categories. One of which is to do it mechanically. Mechanically cleaning entails manually scrubbing the pipes. This type of method, however can be labour intensive, time consuming, can be expensive and has a long down time on supply. The other of these methods is biological cleaning. Biological cleaning is achieved by introducing biological substances into the pipe network that will destroy or eat away the biofilm, this however is dangerous as some of

these biological substances may be unsafe for human consumption and will be hard to completely flush out. Another way to remove biofilms is by altering the environment inside the pipes of the system by controlling the conditions such as pH, temperature, hydrodynamics status and residual chemical composition. If the right environment is kept this will dramatically slow down biofilm growth. Problem is it is costly and hard to maintain such conditions in the pipe systems. The last of the four cleaning methods is to employ chemicals. This needs adequate cleansing before returning to the consumer depending on which chemicals have been made use of. The chemicals employed might also adversely react with the pipe material, giving rise to a new problem.

Silver nanoparticles display a wide range of biocidal activity to many microorganisms like, Fungai, algae, bacteria and viruses (Marambio-Jones & Hoek, 2010). It is because of these properties of silver nanoparticles that its application to eradicate biofilms in water supply pipelines is being investigated. Table 2.1 below gives a brief summary in some of the other available options of biofilm control.

Table 2.1 Biofilm Control Methods

Control Method	Description / Notes
Chemical	E.g. Silver silver nanoparticles may destroy cell membranes because of the negative charge the membrane possesses. (Elechiguerra JL, 2005) This negative charge is called a free radical, an unpaired valence electron. Their extremely reactive free radical takes any chance it gets to bind, ripping apart anything it needs to, including the bacterial membranes. (Kannan N, 2011)
Physical	i) Mechanically scrubbing the piplines ii) Control of environmental growth conditions: To reduce the frequency of biofilm removal by limiting its formation and regrowth, after removal is one way to make the maintenance issue as a whole more cost effective. Limiting biofilm growth is basically making an environment that is not conducive for the biofilms. A) Environmental Factors Temperature of water affects the microbial growth rate most. It has been observed that most microbial growth and activity happens at temperatures higher than fifteen degrees Celsius. (Anon., 1992). B) Sediment Accumulation

	The fines are not detected when breakthrough occurs because of their colour and may carry with them bacteria. The particles accumulate at dead ends and low flow areas of the water distribution network. C) Corrosion Corrosion influences biofilm growth in numerous ways such as, slowing down the water close to surface reducing shearing forces, sheltering biofilms from disinfectant and providing habitats. D) Disinfection Residual Concentrations Low levels of residual disinfectant in water supplies results in the undisturbed growth of biofilms. E) Nutrient Availability Carbon is used for cell generation and can be an energy source. Bacteria (heterotrophic) need these three nutrients in a ratio of; carbon 100: nitrogen 10: phosphorus 1, carbon is usually the limiting one. Carbon sources from natural sources and could enter the system through pipe breaks. F) Hydraulic Effects An increase in flow will increase shearing, and disturb biofilm gowwth. Water flow is altered by fire hydrants, water main breaks, flushing and other factors due to the design of network and pipe size.
Ultrasound	It is well established that ultrasonic cleaning baths, such as the Camlab device operating at 33 kHz, generate sufficient cavitation bubble activity to clean contaminating coatings, deposits and biofilms from metallic, glass, ceramic and plastic surface It has long been established that ultrasound provides an effective means of cleaning surfaces. The use of wide transducers with low surface energies in conventional ultrasonic cleaning baths allows the induction of cavitational bubble activity throughout the tank. It has also been established that extensive bacterial biofilm (10^cfu cm^2) can be cleared from surface in this way.
Shock waves	There has been research on the feasibility of shockwaves as a method of removing biofilms. Results showing that shock waves have the ability to reduce the number of bacteria on a surface have been found (Philip Muller, 2011). Shock waves are able to greatly decrease adherent bacteria to an extent comparable to the control
Biological	Johansen et al. (1997) experimented on steel and polypropylene substrates which consisted of a model a biofilm which has four bacteria family groups: Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas fluorescens and Pseudomonas aeruginosa. Their experimental results concluded that a mixture of polysaccharide-hydrolysing enzymes combined with oxidoreductases was able to remove biofilm from the substrate and stop bactericidal activity.

	The usage of enzymes to remove biofilm is still restricted, due to the very high prices of the chemicals being used and the lack of techniques for quantitative evaluation of the effect of enzymes, as well as the lack of commercial availability of the different enzyme activities, limits their usage (Johansen et al., 1997).

2.2 Silver Nanoparticles (Ag+)

2.2.1 The antimicrobial effects of Silver (Ag) ion.

Silver nanoparticles have displayed strong antibacterial properties and its applications can be but not limited to water and air purification, medical appliances cleansing, food production, clothing and cosmetics (Chen & Stewart, 2000) . Silver nanoparticle applications have been explored in various fields such as pharmaceutical engineering and medicine and has expanded considerably (Martinez-Gutierrez, et al., 2013). In medicine for example silver nanoparticles have been seen to reduce the microbial infections on wounds , in skin and also prevent bacterial colonization of medical instruments (Martinez-Gutierrez, et al., 2013). Silver has also been explored as an antibacterial agent in its many different forms such as stabilized silver salts, silver impregnated zeolite, silver-titanium dioxide composite Nano powders, silver chloride particles, polymer-silver nanoparticle composites, activated carbon materials, silver-coated polyurethane and silver-dendrimer complexes and composites.

Martinez-Gutierrez, et al., (2013) attributes the silver nanoparticle antibacterial effects to their unique physio-chemical abilities such as, high reactivity and high surface area to volume ratio. These characteristics result in silver nanoparticles being used in microorganism resistant drugs and other alternate products. Marambio-Jones & Hoek ,2010, confirms this and further describes the factors affecting the level of toxicity to bacteria of silver nanoparticles to be, shape and size, environmental factors such as pH and ionic strength, surface chemistry, crystallinity and capping agents. Particles with shapes that contain more facets like triangular particles tend to have the most antibacterial properties due to their stability. The stability of the silver nanoparticles has an effect on its effectiveness as antibacterial agent because the formation of aggregates causes a reduction in biocidal activity (Marambio-Jones & Hoek, 2010), as well as the readiness to release silver ions, that are stated by Lok et al., 2008, to be the cause of the antibacterial characteristics of silver nanoparticles. The more stable the chemical is the higher its antibacterial properties.

Silver nanoparticles properties such as shape, ionic strength and particle size lend to its stability and effectiveness (Marambio-Jones & Hoek, 2010).

Silver has antibacterial properties in many different forms apart from metallic silver nanoparticles such as stabilized silver salts, silver impregnated zeolite, silver chloride particles, activated carbon materials, to name a few. These forms of silver are already being utilized in various products (Martinez-Gutierrez, et al., 2013). The release of the silver ion is thought to be the primary reason for these antibacterial characteristics that silver has (Marambio-Jones & Hoek, 2010). Silver antimicrobial characteristics are achieved by three main ways when the silver ion interacts with the cell The release of these ions is then followed by the damaged cell wall structure increasing the cell permeability, cell leakage, loss of the proton motive force and damage to the RNA and DNA (Marambio-Jones & Hoek, 2010). All these following consequences of the ions interacting with the microbial cell then results in the eradication of the bacteria. Details of these three ways are given below.

2.2.1.1 Cell entry

When silver is reduced to the nanoscale it gains charge, this is an important part of the destruction of the bacterial cell membrane. The positively charged silver ions get attached to the cell walls and vacuoles of bacteria, this damages cell envelope and cell wall structure, cytoplasmic membrane, and its contents. Silver nanoparticle ions damage and increase the permeability of the cell membrane. The interaction of the silver nanoparticle with the bacterial cell membrane leaves pits in the membrane when the silver ion enters the cell (Marambio-Jones & Hoek, 2010). This leads to cell leakage and the uptake of external hazards into the cell.

The microbial cell membrane possesses negative charge which is called a free radical. This unpaired valence electron is and will bind with anything it can rapturing anything it needs to reach equilibrium. The silver ions are positively charged and bind with the cell membrane damaging it. (Kannan N, 2011) . silver ions have a high affiliation to phosphorous and sulphur within the cell membrane. This allows the silver nanoparticles to attach to the cell wall and penetrate it leaving pits and pores on the damaged surface (Alexa M. Königs, 2015). The bacteria are now susceptible to cellular leakage, where the cytoplasm pours out into the extracellular fluid carrying with it key intracellular components of the cell (Gurunathan, et al., 2014).

2.2.1.2 Protein inactivation and DNA interruption

The infiltration of silver leads to the positively charged ions disrupting the ATP production and DNA replication (Marambio-Jones & Hoek, 2010). The silver ions are released from the silver

nanoparticles. This occurs in the mitochondria of the cell (Marambio-Jones & Hoek, 2010). The free silver ions interact with the respiratory function enzymes resulting in the deformation of the chain that forms ATP (Marambio-Jones & Hoek, 2010). Silver also the joins to the proteins causing proton leakage. Silver inhibits the uptake of phosphate; this inhibits the DNA from functioning and replicating. The extensive production of reactive oxygen species, which is a by-product of the cellular respiratory mechanism, creates free radicals which attack membrane lipids causing the breakdown of the cell membrane (Marambio-Jones & Hoek, 2010). These high levels also interfere with mitochondria function and DNA damage (Marambio-Jones & Hoek, 2010). Silver acts as a catalyst in the generation of these reactive oxygen species. Silver nanoparticles are also responsible of reducing the levels of glutathione disulphide which an antioxidant which control the levels of reactive oxygen species (Marambio-Jones & Hoek, 2010).

Silver nanoparticles have proved in vitro to be effective in killing the bacteria such as, *Klebsiella pneumonia staphylococcus epidermis, Leuconostoc mesenteroides, Escherichia coli, Klebsiella mobilis and. staphylococcus aureus*, which are the main bacteria found within biofilms found in water pipes (Marambio-Jones & Hoek, 2010). In addition to these bacteria silver nanoparticles have proven to have adverse effects on some Fungai that have been in biofilms such as *Saccharomyces cervisia, Aspergillus, Penicillium citrinum and Candida albicans*. Silver also kills a few viruses such as hepatitis B and syncytial virus (Marambio-Jones & Hoek, 2010)

Martinez-Gutierrez, et al., (2013) tested the antibacterial effects of silver in its nanoparticle form. The biofilms treated were formed in both static and fluid flow conditions. A reduction in bacterial clusters of *Pseudomonas aeruginosa* was obtained. Martinez-Gutierrez, et al., (2013) concluded that silver nanoparticles both prevent the growth of bacteria and remove the biofilms that have already been formed. This makes silver nanoparticles a viable option in the control of biofilm bacteria control.

In the experiment carried out by Huang, et al., (2008), the treatment of microorganisms with silver nanoparticles resulted in the inhibition of the biofilm formation. Huang, et al., (2008) also recorded that p. aeruginosa was the most affected bacteria to the silver nanoparticle treatment and resulted in a log 2 reduction. The anti-bacterial effects of silver nanoparticles against *Staphylococcus epidermidis and E. coli* was also investigated. These above-mentioned bacteria are the most common in aquatic formed biofilms. The overall results of the study carried out by Huang, et al., (2008) show that silver nanoparticles are toxic to bacteria that are mainly found in biofilms. From these results it is noted that higher concentrations of silver nanoparticles are needed to completely kill microbes that are inside biofilms. *Stenotrophomonas maltophilla, Acinetobacter baumannii*

and *Pseudomonas aeruginosa* are all susceptible to silver ions and will be completely eradicated within 3 day (Huang, et al., 2008) s.

In the research by Sheng, et al., (2015) silver nanoparticles were observed inside the biofilm after only forty-five minutes of exposure. Within 24h the silver nanoparticles had infiltrated the microbial cells and started killing them off. Sheng, et al., (2015) tested the power of silver nanoparticles on waste water formed bacteria. Silver nanoparticle have harmful effects even on the wastewater biofilm bacteria (Sheng, et al., 2015). Apart from the direct killing of the microbial cells by the silver nanoparticles they also inhibit nitrification even at low concentrations of about 1mg Ag/L (Sheng, et al., 2015).

Bouryabaf, et al., (2017) researched the efficacy of agar hydrogel with silver nanoparticles to remove biofilms and kill bactreia, mainly *Staphylococcus aureus.* From the reuslts it can be concluded that, silver nanoparticles were essentail in the killing the bactria and can be used as an effective antibacterial agent. Silver nanoparticves attack the bacterial cell mebrane, interact with theprotiens containing sulfur, and cause disruption and alter phophorous containing compounds like the celluar DNA (Bouryabaf, et al., 2017). Silver nanoparticles have been seen to have adverse effects on , *Escherichia coli, Listeria monocytogenes, pseudomanas aeruginosa, klebsiella pneumoniae and proteus mirabilis* (Bouryabaf, et al., 2017).

Thurman, et al., (1989) reported that it's the concertration of silver oions that is respoincble for its antibacterial properties and not its physical nature. Silver ions are known to be toxic to E coli , Bacillius typosus, Staphylococcus Pseudomonas , Streptococcus and Salmonella (Thurman, et al., 1989). This list contains bacteria that are resistant to antibiotics and some viruses are also subject to it effect.

2.2.2 The disadvantages of using Silver (Ag) as an antibacterial agent.

Marambio-Jones & Hoek (2010) reviewed that the use of silver nanoparticles has no documented immediate impacts to human health or the environment, although more research is needed before implementation of any silver nanoparticle product. Bacteria can develop resistance or become less sensitive to toxicants by natural selection in environments where these antimicrobials are present. The wide use of silver in many forms may have cultivated some silver resistant bacteria (Marambio-Jones & Hoek, 2010).The bacterial resistance to silver and other heavy metals is mainly encoded within the plasmid genes of the bacteria, but chromosomes could also encode silver resistant genes (C Baker, 2005). The increasing population of resistant bacteria makes

uncertain the efficacy of silver nanoparticles as an antimicrobial agent. The presences of halides such as chloride, bromide and iodide, can decrease the silver bioavailability and by so doing reduce the exposure of silver to bacteria (Chen & Stewart, 2000). Minimising the bacteria exposure to silver increases its effectiveness when its applied for antibacterial purposes.

Allot of silver containing products are being developed. This however calls for more research into its effects and risks on humans and to the environment. Although silver has its advantages of being toxic to bacteria the assessment of its harmful potential to humans and the ecosystem associated with its utilization need to be investigated further Marambio-Jones & Hoek (2010) states. Prokaryotic and eukaryotic cells are different but understanding the ways in which silver kill bacterial cells, one can see the potential of silver nanoparticles to hurt even mammalian cells. Silver nanoparticles attack cell components such as the mitochondria which are present in both prokaryotic and eukaryotic cells. The fundamentals of mitochondrial function are the same in both bacterial cells and mammalian cells (Marambio-Jones & Hoek, 2010). Functions that are essential to the cell survival like, ATP synthesis, electron transport, respiration and proton motive force, are like prokaryotic and eukaryotic cells. In theory, it is thought that silver could affect the function on higher cellular organisms via the same ways it kills bacterial cells, if the silver can enter the cell (Marambio-Jones & Hoek, 2010). Human exposure to silver nanoparticles would be mainly through the respiratory system, digestive system and through the skin. In addition, other ways in which silver exposure can occur are by being exposed to wounds, through wound dressing and antibacterial products and the female genital tract, through hygienic, and contraceptive products. In addition to silver anti-bacterial effects Marambio-Jones & Hoek (2010) reviews that silver can also have negative effects in higher cell species like zebra, rodents and fish. In rodents silver damages the internal organs such as the lungs and liver, and silver also penetrates the blood brain barrier (Marambio-Jones & Hoek, 2010). One study in human cells showed that silver can be genotoxic (C Baker, 2005).

2.2.3 Silver nanoparticle synthesis

Kamali (2011) investigated the synthesis of silver nanoparticles using chemical reduction, using Silver nitrate, citric acid and sodium hydroxide. This investigation resulted in the formation of silver nanoparticles proving the efficacy of citric acid as a complexing agent. Kamali (2011) found that addition of ammonium hydroxide have effects on the particle size but the particle purity is increased when nitric acid is introduced. The purity, size and morphology of silver nanoparticles

strongly affect there chemical and physical properties and as such different approaches of synthesising them have been adopted. Namely, microwave irradiation, chemical deduction, green synthesis, gamma irradiation and thermal methods (Kamali, 2011).

Huang, et al., (2008) sythesised silver ions using silver chloride dissolved in ionised water. The most common method of synthesising silver nanoparticles is a chemical reaction by which a dissolved silver salt is reduced by a compound such as citrate, ascorbate, glucose and hydrazine (Marambio-Jones & Hoek, 2010). Grouchko, et al., (2009) used silver anaoparticles that where sythesised by a chemical method reducing silver nitrate by trisoduim citrate.

2.3 Copper Nanoparticles (Cu^{2+})

2.3.1 The antibacterial effects of Copper. (I and II)

Cooper ions have been used to eradicate bacteria in hospital water reticulation systems. Copper ions have been recorded to inactivate *Legionella pneumophila* under 3hours with concentrations of about 0.1mg/l (LIN, et al., 1996). Copper has also been employed as an algicide (Thurman, et al., 1989). It is one of the most effective in killing heterotrophic bacteria in water-based environments. Thurman, et al., (1989) discovered that copper at 0.2 mg/L inactivates the infectious virus, bronchitis in only two hours. It is also very toxic to E coli. Hassen, et al., (1998) tested the effects of copper on *Pseudomonas aeruginosa* and bacillus thuringinesis and noted that at 0.2mM inhibited bacterial growth. The intric properties of copper and effect on the bacteria cell wall were credited for the antibacrtail charateristics displayed by the copper. Bacillus thuringinesis however did not have as significant reduction when exposed to copper as compared to other heavy metals (Hassen, et al., 1998) Copper ion totally eradicates *Pseudomonas aeruginosa* when its treated with copper (Huang, et al., 2008). *Pseudomonas aeruginosa* is more susceptible to copper ion treatment compare to Stenotrophomonas maltophilla and *Acinetobacter baumannii.*

The microbial killing effects of copper is closely in connection with oxidation of the sulfhydryl groups of enzymes with in the bacterial cell (Pyle, et al., 1992). The oxidation of these enzymes inhibits the enzymes activity (Pyle, et al., 1992). The enzymes inhibition affects the raspatory function of the cell, thus killing the bacterial cell.

2.3.2 The disadvantages of using Copper (Cu) as an antibacterial agent.

Humans are exposed to Copper in varies ways in day to day living, and the known effects of chronic excess copper exposure on health is insufficient (Araya, et al., 2005). Copper is one of the necessary micronutrients needed in many processes of life, but can be toxic and harmful to cell membranes, proteins and DNA if exposed to high amounts. High levels of copper have been seen to collate with Wilson's disease (Araya, et al., 2005). Test was carried out by Araya, et al., (2005) exposing various gender and age groups of people to a daily dose of 10mg Cu/day for 60 days. Observed was enzymes in the liver increased greatly in all test samples but still below clinical liver dysfunction levels.

In other studies, it was found that foetus development is affected negatively by the deficits of copper, and excessive exposure is also risky (Uriu-Adams & Carl, 2005). Liver disease and severe neurological defects are some of the additional results of chronic copper exposure (Uriu-Adams & Carl, 2005). Tissue damage is also caused by oxidative stress by both copper deficiency and toxicity (Uriu-Adams & Carl, 2005). The oxidative stress caused by the high levels of copper is because of its redox reactivity. The copper could also attach to the free this of cysteines. This causes oxidation and crosslinks that impair protein activity (Uriu-Adams & Carl, 2005). Many cases recorded in literature report toxicity due to ingestion of liquids that have been contaminated with copper. Symptoms of chronic copper exposure include nausea, vomiting, abdominal pain, liver and kidney failure, headache, respiratory difficulties and gastrointestinal bleeding. Copper toxicity could occur through the skin and respiratory tract of a human (Uriu-Adams & Carl, 2005).

Wilson's disease is a genetic disorder disease caused by copper. It's an autosomal recessive disease and has a heterozygous carrier rate of about 1:100, with an estimated prevalence of 1:30 000 (Uriu-Adams & Carl, 2005). The overload of copper that happens within the body results with liver pathogenesis that is a consequence of Wilsomkin's disease. Liver failure causes death. Alzheimer disease, Amyotrophic lateral sclerosis and Creutzfeldt-Jakob disease are connected to the high amounts of copper that accumulate in regions of the brain.

2.3.3 Copper nanoparticle synthesis

Chemical reduction method of synthesising copper nanoparticles is one of the most widely used do to its ability to vary the attributes. such as size purity morphology and stability, of the particles to the user's needs (Grouchko, et al., 2009). Reducing agents used in chemical methods have to

be strong, for example, hydrazine, sodium borohydride (Grouchko, et al., 2009), hydrogen amongst many.

Grouchko, et al., (2009) achieved well dispersed copper nanoparticles by reducing copper nitrate solution using hydrazine monohydrates as a reducing agent. This was carried out in the presence of silver nanoparticles to catalyse the reaction. In this investigation it was found that the rate of formation of copper nanoparticles was significantly by the silver nanoparticles. In other studies Huang, et al., (2008) sythesised copper ions using copper choride dissolved in ionised water and Jadhav, et al., (2011) prepared copper oxide ananoparticles by electrochemical reduction. Tetra butyl ammonium bromide was used in this sythesis as a structure directing agent.

2.4 Combined Effects of Silver and Copper

2.4.1 Antibacterial effects of heavy metals

Heavy metal group which contains copper and silver includes other transition series metals from the periodic table. Within the metallic element groups there are heavy metals such as zinc, mercury, bismuth, cadmium, chromium, copper and silver that show antibacterial characteristics (Top & Ulku, 2004). These heavy metals react with and impart their antibacterial properties onto zeolites. Heavy metals been recorded to be harmful to *Pseudomonas aeruginosa* and Escherichia coli (Top & Ulku, 2004). Zinc oxide nanoparticles PVC composite surfaces reduces Staphylococcus aureus biofilm formation. Silver and copper are both heavy metals. Heavy metals have antibacterial properties and are used as antibacterial agents (LIN, et al., 1996). Hassen, et al., (1998) confirms that heavy metals are toxic to bacteria. Mercury and copper have been found to the elements that affect *Pseudomonas aeruginosa* and bacillus thuringinesis the most after testing the six most readily avalable metals, namely; copper, zinc, cadium,chromium, mercury and coblat.

2.4.2 Synergistic antibacterial effects of Silver and Copper

The antibacterial characteristics of silver and copper ions is heavily attributed to their positive ions. These ions are microbiocidal because of their charge they attached to the bacteria cell wall and increase the cell wall permeability (LIN, et al., 1996). This leads to cell leakage and interference with proteins inside the cell and results in cell death. There have been many

publications that document the use of Copper and silver ions in control of bacteria, viruses, algae and fungi in water (Pyle, et al., 1992). Both silver and copper ions interfere with the enzyme's activities of the cell, inhibiting critical cellular function such as respiration. Although chlorine, which is the most common antibacterial agent used in purifying water , has quicker antibacterial action, there is an synergetic effect when its used in combination with silver and copper (Pyle, et al., 1992). The combination or individual applications of silver and/or copper ions is being used in over 300 hospitals in the world to purify their portable water systems (Huang, et al., 2008). The efficacies of silver and copper ions to eradicate bacteria has been proven a number of times (Huang, et al., 2008). They have been effective in killing *Legionella, Pseudomonas cepacia, Naegleria fowleri and Poliovirus*. Copper and silver ions have a synergetic effect when they are used to treat *Pseudomonas aeruginosa* and *Acinetobacter baumannii.*

The combined effects of treating *Legionella pneumophila*, a bacteria found in water reticulation systems, with both copper and silver ions yielded more favourable results than using the two chemicals separately (LIN, et al., 1996). Lin, et al., (1996) observed that the concentrations of silver ions and copper ions needed to kill the bacteria individually were more than double compared to the concentration of the mixture of the two chemicals to achieve the same result. Lin, et al., (1996) also showed that synergy between silver and copper ions exists.

The combination of silver and copper nanoparticles has been tested and resulted in the reduction of bacterial cells (Pyle, et al., 1992) far quicker than either of the two elements alone. Pyle, et al., (1992) also tested silver and copper antibacterial effects in combination with another halogen, iodine. Observed was that the presence of the metal enhanced the action of the halogen antibacterial effects (Pyle, et al., 1992), and as with chlorine the combination yielded much better results than the individual treatments . The combination of copper and silver with a halogen would be best suited in area where a high count of coliform bacteria have been located in water distribution networks (Pyle, et al., 1992). The one down fall of using these metals is that they tend to gravitate towards the surface leaving small amounts of metal ions in the liquid form (Pyle, et al., 1992). However, they reduce and inhibit the formation of biofilms in pipelines. They also make halogen disinfections more effective in places where biofilms have already taken root in systems where biofouling is a problem.

3. Methodology

In a preceding study, biofilm bacterium was exposed to silver nanoparticles for various periods in 1mg and 2mg (per 25ml) concentrations. The results displayed a natural log delay with the required bacterial count being obtained at day 7. The research herein, was aimed at investigating the reduction of the effective time, the synergetic effects of copper nanoparticles and the application of nanoparticles in flow conditions. Concentrations of 10mg, 20mg and 50mg (per 25ml) of silver and copper nanoparticles were utilized in beaker and channel flow experimental setup varying the exposure time. A combination of copper and silver nanoparticles with concentrations 5mg ,10mg and 50mg were also tested in similar conditions to investigate the synergetic effects of the two heavy metals. Grouchko, et al., (2009) used silver nanoparticles that where synthesised by a chemical method by reducing silver nitrate by trisoduim citrate. Grouchko, et al., (2009) achieved well dispersed copper nanoparticles by reducing copper nitrate solution using hydrazine monohydrates a reducing gent. This was carried out in the presence of silver nanoparticles to catalyse the reaction. In this experiment the silver nitrate and copper nitrate were reduced by citric acid. Mortar sample coupons were used because eThekwini uses steel pipes with mortar lining for clean water delivery. Below the description of each stage of the experiment is given.

3.1 Biofilm Cultivation

3.1.1 Mortar sample

Cement mortar mix to fabricate the samples was made using these guidelines:

- Ordinary Portland Cement (OPC) complying with requirements of SABS 471/SANS 50197-1(new code)
- Water used shall be free from injurious amounts of acids, alkalis or substances that may impair the strength or durability of the cement mortar lining
- The aggregate shall be specially graded washed silica sand complying with the grading requirements of SABS 1090 table1 column3, and shall comply with SABS 1083/SANS 1083(new code) in respect of organic impurities and dust content
- Cement, aggregate and water can be mixed to an accuracy of 20% of the mass required. All mortar shall be mixed in suitable mortar mixers. No hand mixing to be permitted
- The total content of cementitious materials shall not be less than 600kg/m^3
- The total water content shall be the minimum required to produce a suitable consistency for application of the lining, but water: cement ratio shall not exceed 0.45:1 by mass

- The finished surface shall be free of excessive laitance, the maximum acceptable thickness being 10% of the total lining thickness or 1.25mm, whichever is the lesser

The concrete mortar sample made is about 10mm by 5mm as seen in Figure 3.1 below.

Figure 3.1: Concrete coupon samples

3.1.2 Growth incubation

The concrete samples were placed in a growth tank with flowing tap water and covered to block the sunlight. The tank was kept in a cool area so to mimic the underground pipe conditions as seen in Figure A.6 in appendix A

Samples were left in the tank for over 150 days to allow adequate growth on the samples.

3.2 Beaker Set-up

Samples were exposed to the chemicals for different periods in stagnant solution in beakers. Table 3.1 summaries this procedure. 3 sets of control samples were taken from the growth tank and preserved without treatment for each tested time period. Nine solutions of chemicals were prepared as shown in Figure 3.2.

Figure 3.2:Prepared nanoparticle chemicals

Figure 3.3: Treated samples in beakers

Table 3.1: Beaker set-up sample treatment summary

(0L/s flow)	3HRS			12HRS			24HRS		
Silver	10mg	20mg	50mg	10mg	20mg	50mg	10mg	20mg	50mg
	(3 samples)	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*
	10mg	20mg	50mg	10mg	20mg	50mg	10mg	20mg	50mg

Copper	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*
Silver + Copper	5mg	10mg	50mg	5mg	10mg	50mg	5mg	10mg	50mg
	(3 samples)	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*
Control	0mg			0mg			0mg		
	(3 samples)			*(3 samples)*			*(3 samples)*		

3.3 Channel Set-up

3.3.1 Design

The channel apparatus was designed to test effects of the flow and the chemical concentration simultaneously. The detailed design can be found in Appendix A, Figure A.8 and Figure A.9.

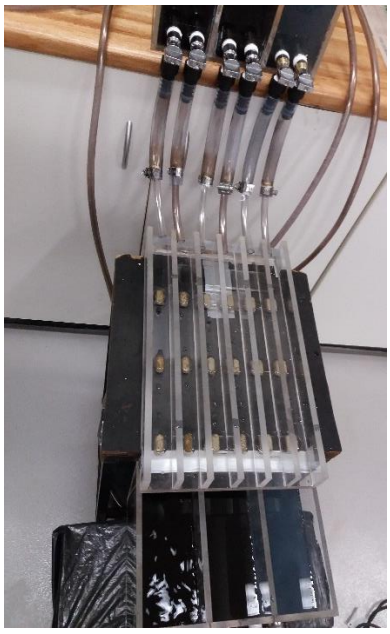

Figure 3.4: Channel set-up experiment

The supply and collection tanks have 3 sections as shown in figure 3.4. Each section is connected to 2 channels. Each channel has an adjustable tap to regulate the flow. Each channel has 3 slots to place samples.

The channel experiment is open flow and has very little flow as compared to the high pressured pipelines used in distribution networks. Although these conditions may be different it is a good investigation to how the nanoparticles behave in moving water as supposed to the stationary conditons application of the beaker experiments.

3.3.2 Procedure

The samples were transferred from the growth tank into the channel slots for each of the 3 tests. The 3 tests were done with 3 different chemicals. Silver nanoparticles, Copper nanoparticles and a mixture of Silver and copper nanoparticles. Each test had 3 different nanoparticle concentrations. The samples were subjected to 2 different flows of the same concentration. A control group was taken from the growth tank at the beginning of each test and preserved for processing. Samples were tested in triplicates. All tests were run for 12H. The Table 3.2 below illustrates the above procedure.

Table 3.2: Channel set-up sample treatment summary

(12hrs run time)	FLOW 1			FLOW 2			CONTROL
Silver	10mg	20mg	50mg	10mg	20mg	50mg	0mg
	(3 samples)	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*
Copper	10mg	20mg	50mg	10mg	20mg	50mg	0mg
	(3 samples)	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*
Silver + Copper	5mg	10mg	50mg	5mg	10mg	50mg	0mg
	(3 samples)	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*	*(3 samples)*

The average flows of each channel during each test are recorded below in Table 3.3. The recorded flows can be found in Appendix A

Table 3.3: Flow rates during channel set-up experiment

Ave Flows of Channel Exp.		
Silver	Fast Flow	Slow Flow
	0.0191	0.0068
Copper	Fast Flow	Slow Flow
	0.0123	0.0059
Silver + Copper	Fast Flow	Slow Flow
	0.0105	0.0059

3.4 Chemical Synthesis

The ratio of Silver Nitrate and Copper Nitrate to Citric Acid to give the required nanoparticle concentrations are:

Ratio of Silver (Ag) : Citric - 1: 2.47

(Cu) : Citric - 1: 2.47

Kamali, (2011), had ratio of 1.7 : 4.2. The same procedure was taken but calculations were done from first princeples by making use of the formlua:

M = **V** x **1/MM**m x **MM**s x **Ratio**

(mass) (volume) (molar mass metal) (molar mass of salt)

The masses need to be dissolved in distilled water to make the desired concentrations are noted in table form below in Table 3.4 to Table 3.9.

3.4.1 Analytical Reagent Synthesis

Sodium Hydroxide was used as the analytical reagent. 1 molar of this solution was added to the chemical mixtures as stipulated in the tables below. The 1 molar solutions were made as follows:

	NaOH (mass)	Water (distilled)
Sodium Hydroxide (1M)	20g	500ml
	40g	1000ml

3.4.2 Silver nanoparticle synthesis

Table 3.4: Silver nanoparticle synthesis (250ml solution)

Silver nanoparticle - 250 ml solution – Beaker setup			
NANOPARTICLE CONCENTRATION	CHEMICAL		MASS (mg)
10mg/25ml	Silver Nitrate	$AgNO_3$	157.50
	Citric Acid	$C_6H_8O_7.H_2O$	389.03
20mg/25ml	Silver Nitrate	$AgNO_3$	314.98
	Citric Acid	$C_6H_8O_7.H_2O$	778.00
50mg/25ml	Silver Nitrate	$AgNO_3$	787.50
	Citric Acid	$C_6H_8O_7.H_2O$	1 945.10
1M	Sodium Hydroxide	NaOH	50ml added to all the solutions and heated at 250

Table 3.5: Silver nanoparticle synthesis (5000ml solution)

Silver nanoparticle - 5000 ml solution – Channel setup			
NANOPARTICLE CONCENTRATION	CHEMICAL		MASS (mg)
10mg/25ml	Silver Nitrate	$AgNO_3$	3 149.80
	Citric Acid	$C_6H_8O_7.H_2O$	7 780.00
20mg/25ml	Silver Nitrate	$AgNO_3$	6 299.60
	Citric Acid	$C_6H_8O_7.H_2O$	15 560.00
50mg/25ml	Silver Nitrate	$AgNO_3$	15 749.10
	Citric Acid	$C_6H_8O_7.H_2O$	38 900.00
1M	Sodium Hydroxide	NaOH	1000ml added to each solution and heated at 250

3.4.3 Copper nanoparticle synthesis:

Table 3.6: Copper nanoparticle synthesis (250ml solution)

Copper nanoparticle - 250 ml solution – Beaker setup			
NANOPARTICLE CONCENTRATION	CHEMICAL		MASS (mg)
10mg/25ml	Copper Nitrate	$Cu\ (NO_3)_2$	189.60
	Citric Acid	$C_6H_8O_7.H_2O$	468.40
20mg/25ml	Copper Nitrate	$Cu\ (NO_3)_2$	379.29
	Citric Acid	$C_6H_8O_7.H_2O$	936.85
50mg/25ml	Copper Nitrate	$Cu\ (NO_3)_2$	948.20
	Citric Acid	$C_6H_8O_7.H_2O$	2 342.10
1M	Sodium Hydroxide	NaOH	50ml added to each solution and heated at 250

Table 3.7: Copper nanoparticle synthesis (5000ml solution)

Copper nanoparticle - 5000 ml solution – Channel setup			
NANOPARTICLE CONCENTRATION	CHEMICAL		MASS (mg)
10mg/25ml	Copper Nitrate	$Cu(NO_3)_2$	3 792.90
	Citric Acid	$C_6H_8O_7.H_2O$	9 368.40
20mg/25ml	Copper Nitrate	$Cu(NO_3)_2$	7 585.80
	Citric Acid	$C_6H_8O_7.H_2O$	18 736.90
50mg/25ml	Copper Nitrate	$Cu(NO_3)_2$	18 964.40
	Citric Acid	$C_6H_8O_7.H_2O$	46 842.10
1M	Sodium Hydroxide	NaOH	1000ml added to each solution and heated at 250

3.4.4 Silver + Copper nanoparticle synthesis

Table 3.8: Silver and Copper nanoparticles mixture synthesis (250ml solution)

Silver + Copper nanoparticle - 250 ml solution – Beaker setup			
NANOPARTICLE CONCENTRATION	CHEMICAL		MASS (mg)
5mg/25ml	Silver Nitrate	$AgNO_3$	78.75
	Copper Nitrate	$Cu(NO_3)_2$	94.80
	Citric Acid	$C_6H_8O_7.H_2O$	428.67
10mg/25ml	Silver Nitrate	$AgNO_3$	157.50
	Copper Nitrate	$Cu(NO_3)_2$	189.60
	Citric Acid	$C_6H_8O_7.H_2O$	857.30
20mg/25ml	Silver Nitrate	$AgNO_3$	314.98
	Copper Nitrate	$Cu(NO_3)_2$	379.30

	Citric Acid	$C_6H_8O_7.H_2O$	1 714.90
50mg/25ml	Silver Nitrate	$AgNO_3$	787.50
	Copper Nitrate	$Cu\ (NO_3)_2$	948.20
	Citric Acid	$C_6H_8O_7.H_2O$	4 287.20
1M	Sodium Hydroxide	NaOH	50ml added to each solution and heated at 250

Table 3.9: Silver and Copper nanoparticles mixture synthesis (5000ml solution)

Silver + Copper nanoparticle - 5000 ml solution – Channel setup			
NANOPARTICLE CONCENTRATION	CHEMICAL		MASS (mg)
5mg/25ml	Silver Nitrate	$AgNO_3$	1 574.90
	Copper Nitrate	$Cu\ (NO_3)_2$	1 896.50
	Citric Acid	$C_6H_8O_7.H_2O$	8 574.40
10mg/25ml	Silver Nitrate	$AgNO_3$	3 149.80
	Copper Nitrate	$Cu\ (NO_3)_2$	3 792.0
	Citric Acid	$C_6H_8O_7.H_2O$	17 148.50
20mg/25ml	Silver Nitrate	$AgNO_3$	6 299.60
	Copper Nitrate	$Cu\ (NO_3)_2$	7 585.80

	Citric Acid	$C_6H_8O_7.H_2O$	34 296.90
50mg/25ml	Silver Nitrate	$AgNO_3$	15 749.10
	Copper Nitrate	$Cu\ (NO_3)_2$	18 964.40
	Citric Acid	$C_6H_8O_7.H_2O$	85 742.30
1M	Sodium Hydroxide	NaOH	1000ml added to each solution and heated at 250

3.5 SEM Processing of Images

The preparation procedure for testing & imaging samples has 3 processes after the sample has been extracted from the experiment.

3.5.1 Preservation:

Extracted samples are preserve in 2.5% Glutaraldehyde (can be refrigerated for a minimum of 2 hours up to 2 weeks). The purpose of glutaraldehyde is to stabilize the sample and neutralise all metabolic activity of the biofilm on the sample. After a minimum of 2 hours, the sample can then be unrefrigerated and thereafter transited to the laboratory. It should be transferred to the lab fully submerged in the fixative solution.

3.5.2 Dehydration:

The dehydration procedure is as follows:

1. Buffer wash using 2.5% Glutaraldehyde for 3 x 5 min
2. Submerge in 30% Alcohol for 2 x 5 min
3. Submerge in 50% Alcohol for 2 x 5 min
4. Submerge in 75% Alcohol for 2 x 5 min
5. Submerge in 100% Alcohol for 2 x 10 min

After dehydration the samples need to be left overnight in a dark room to air dry.

The above process dehydrates the samples using various concentrations of alcohols to enable the samples to be used in the SEM. The dehydration process extracts all water from the sample, both surrounding the samples and in the pores of the material of the samples. It also eliminates any further biotic activity on the samples.

Once the samples have been air dried, they are then be gold plated. The samples are gold plated using a plasma gold plating device. The samples are coated with 2 layers of gold particles to enable conduction of the electrons in the SEM and the sample.

3.5.3 SEM Process:

A scanning electron microscope (SEM) is a form of electron microscope that draws images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, emitting various signals that contain information about the sample's surface topography and composition.

Once the desired position is produced, an image is captured storing all the information in the data attached to the image. A minimum of 5 images need to be captured per sample to allow for an acceptable level of accuracy. A variety of positions on the sample need to be surveyed.

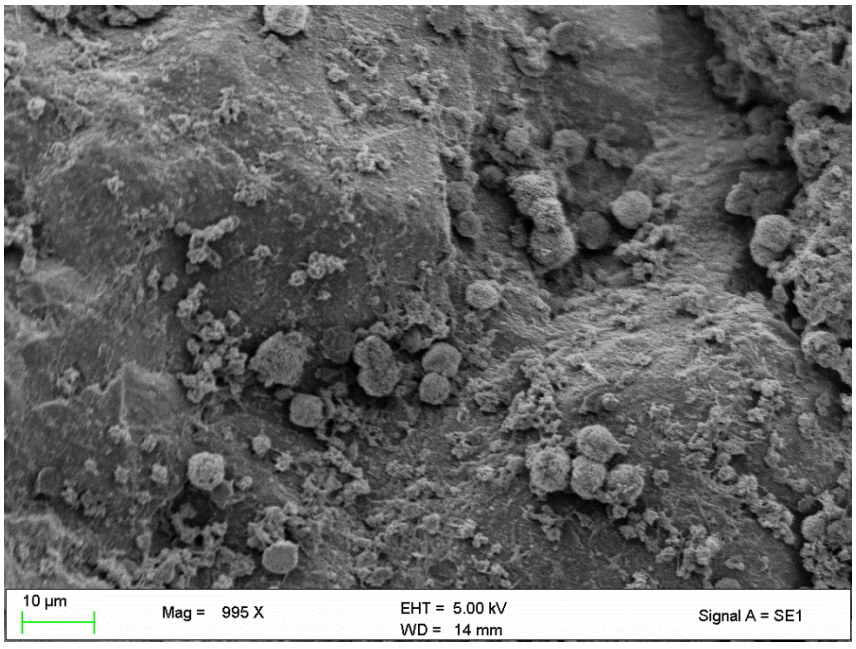

Figure 3.5: SEM image showing bacteria

4. Results and Discussion

4.1 Overview

From the 51 samples used, a total of 765 images were taken from the SEM and processed. Within those there were 6 control groups. From this large pool of data various comparisons can be drawn. The beaker setup primarily examines the different effectiveness of the different concentrations of each chemical used. It also gives data into the combined effect of the Silver and Copper nanoparticles. The channel setup gives the effects of flow on the treatment of the bacteria using these chemicals. The beaker experiment acts as the zero-flow condition for the channel setup. From the results of both setups, as seen in the summary in Figure 4.10, an effective time can be determined.

4.2 Biofilm Growth

The samples were left in the growth tank for approximately 153days before the first samples were taken out for treatment.

4.2.1 Control sample bacterial growth

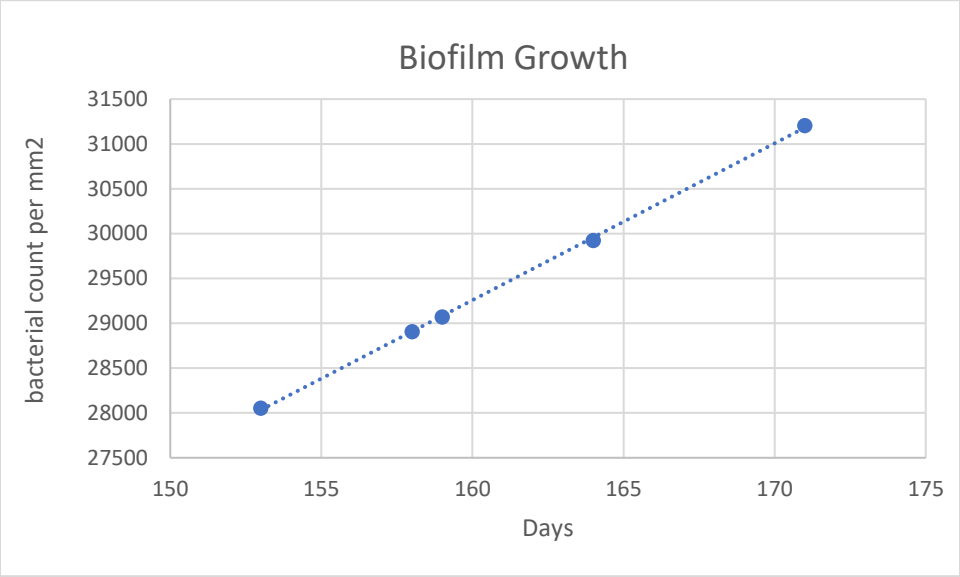

Figure 4.1 Biofilm bacteria average growth from control samples

An average biofilm growth pattern was determined from the control group samples. These control groups were taken out of the growth tank at the specified dates, as in Figure 4.1, and imaged without any exposure to the silver nor copper nanoparticles.

4.3 Beaker Setup Experiment

4.3.1 Silver (Ag) nanoparticles Treatment

Samples were exposed to different concentrations of silver nanoparticles for different periods. Figure 4.2 shows the reduction in bacterial count on the surfaces of these samples, obtained in comparison with the control samples taken out at the time of testing of each experiment.

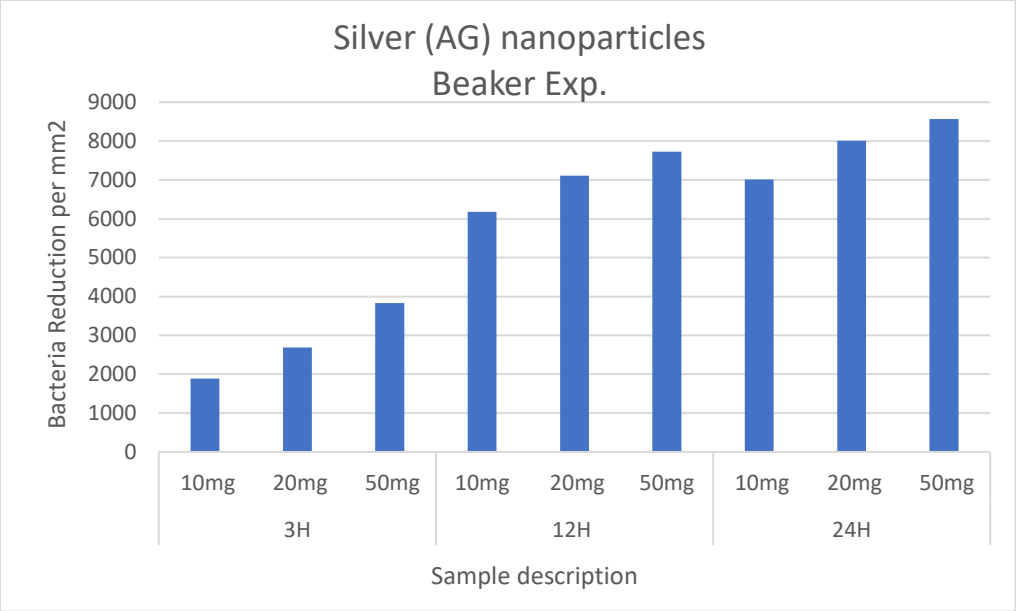

Figure 4.2: Beaker set-up Silver nanoparticle treatment results – effects of exposure period

Generally, as expected the higher concentrations of silver nanoparticles had larger reduction in biofilm surface bacteria. Similarly, the longer periods yielded higher reduction in the bacterial count as well. However, it can be noted from the graph that the ideal exposure time is just over 12 hours, as there is not much difference between the 12hour and 24hour results. The optimum concentration from these set of results can be thought to be around 20mg per25ml as there is no significant jump in bacteria reduction between the 20mg and 50mg concentration results. The 20mg 12hour result is the most optimum in this data set. Figure 4.3 below shows the results set out to compare the effects of the concentrations.

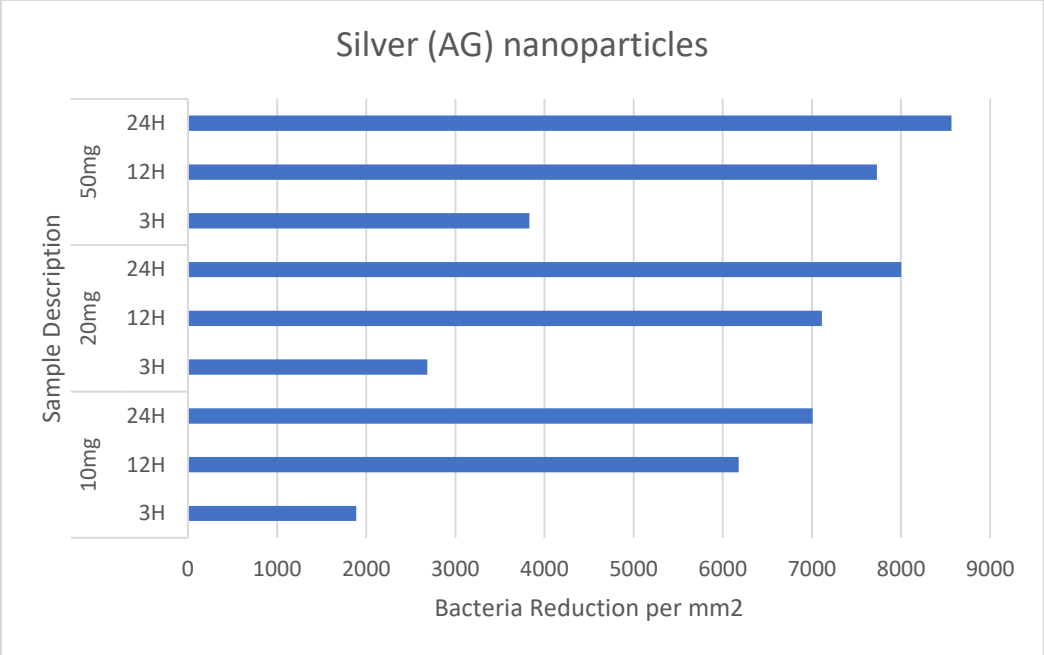

Figure 4.3: Beaker set-up Silver nanoparticle treatment results – effects of concentration

4.3.2 Copper (Cu) nanoparticles Treatment

Samples were exposed to different concentrations of copper nanoparticles for different periods. Figure 4.4 shows the reduction in bacterial count on the surfaces of these samples, obtained in comparison with the control samples taken out at the time of testing of each experiment.

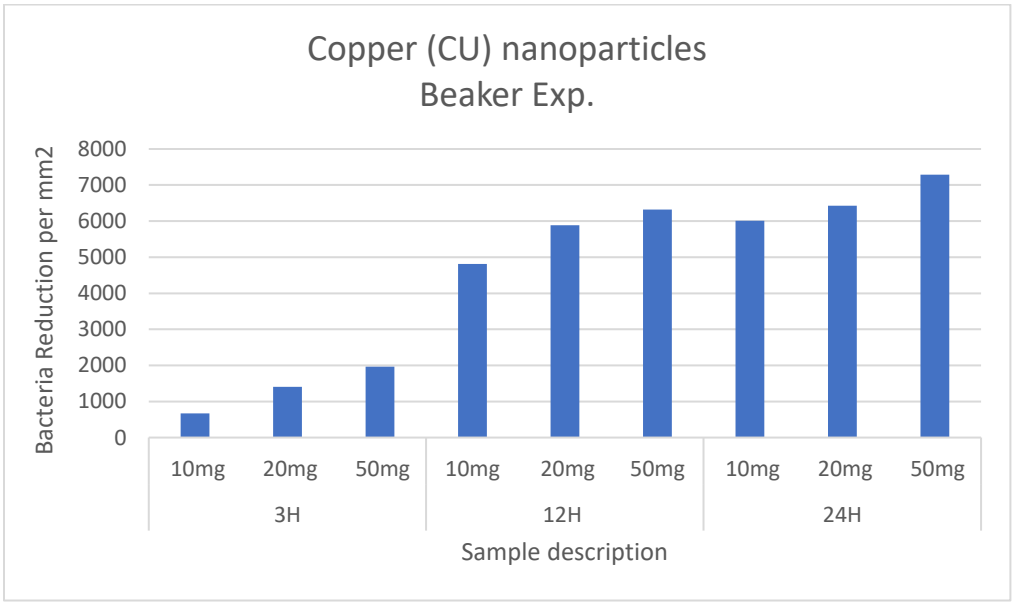

Figure 4.4: Beaker set-up Copper nanoparticle treatment results – effects of exposure period

As in the silver nanoparticle beaker experiment, the higher concentrations of silver nanoparticles and the longer periods yielded higher reduction in the bacterial count. The difference between the 3hour and 12hour copper nanoparticle beaker experiment, shows a significant increase of over 4000per mm^2 bacterial count. This indicates copper nanoparticles need more time to react with the cell membranes or take longer penetrate the biofilm layer. With this set of results, the 50mg 24hour experiment would be the optimum solution and exposure time to get the desired reduction. Figure 4.5 below shows the results set out to compare the effects of the concentrations.

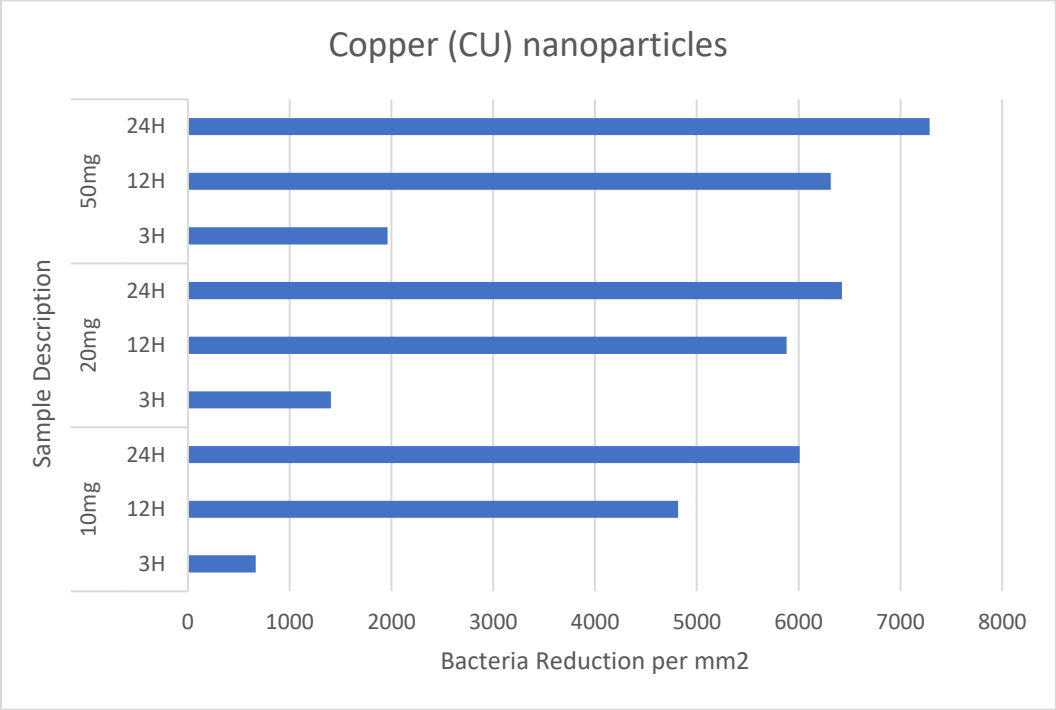

Figure 4.5: Beaker set-up Copper nanoparticle treatment results – effects of concentration

4.3.3 Combined Treatment of Silver and Copper (Ag+Cu)

Samples were exposed to different concentrations of combined silver and copper nanoparticles for different periods. Figure 4.6 shows the reduction in bacterial count on the surfaces of these samples, obtained in comparison with the control samples taken out at the time of testing of each experiment.

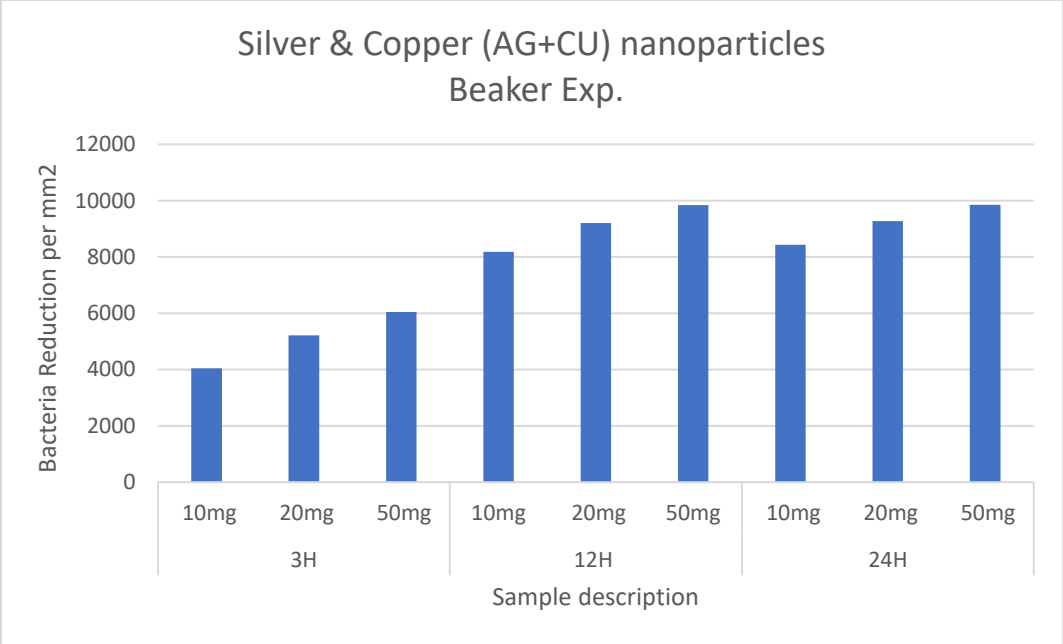

Figure 4.6: Beaker set-up Silver and Copper nanoparticle mixture treatment results – effects of exposure period

In the combined chemical experiment, the overall trend is similar to that of the 2 individual experiments. In short, the higher concentrations and longer periods produce higher reduction in the surface bacterial count on the samples. However, the 24hour experiment seemed to be less effective than the 12hour experiments. The 12hour 50mg experiment having noticeably similar reduction than that of the 50mg 24hour experiment going. The results of the combined chemicals do not have as big differences between the different exposure periods. The optimum experiment of this data set would be the 12hour 50mg concentration of the combined chemicals. The synergetic effects of these 2 chemicals are effective within the short time, and thus the results in the 24hour experiment is most likely a result of the fact that by the 24th hour most of the bacteria have been killed. This trend follows the natural decade logarithm. Figure 4.7 below shows the results set out to compare the effects of the concentrations.

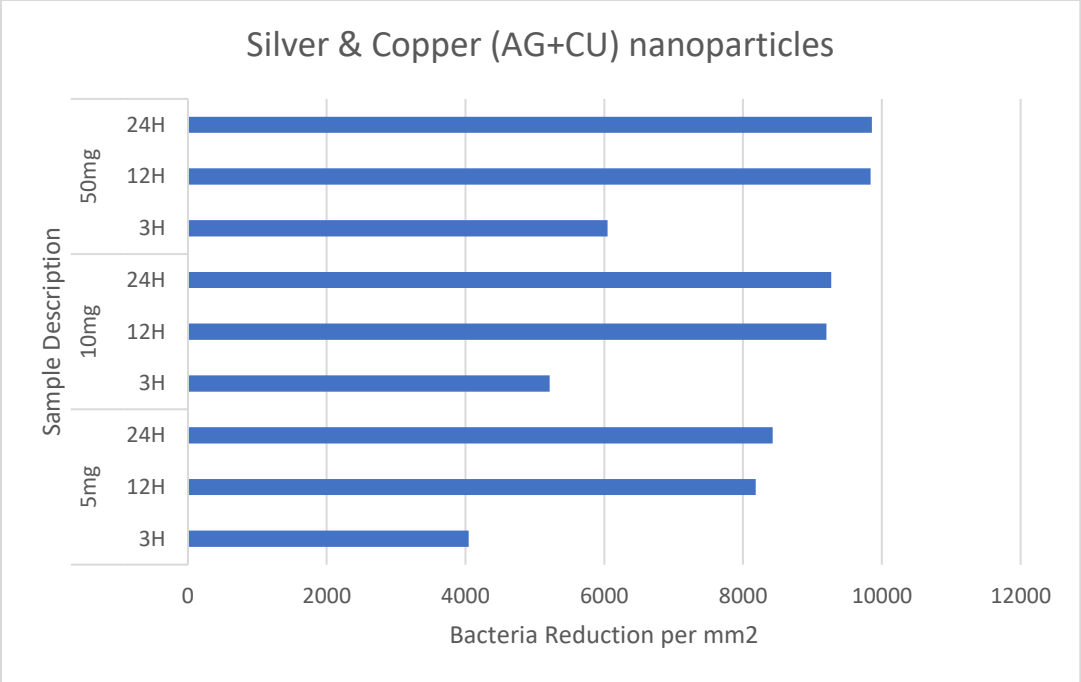

Figure 4.7: Beaker set-up Silver and Copper nanoparticle mixture treatment results – effects of concentration

4.4 Effective Concentration and Effective Exposure Time.

The beaker setup experiment results are combined below in Figure 4.8. The comparison between the silver, copper and combined experimental results can lead to the determination of the effective concentration of each and combined chemicals and the most effective exposure time of treatment.

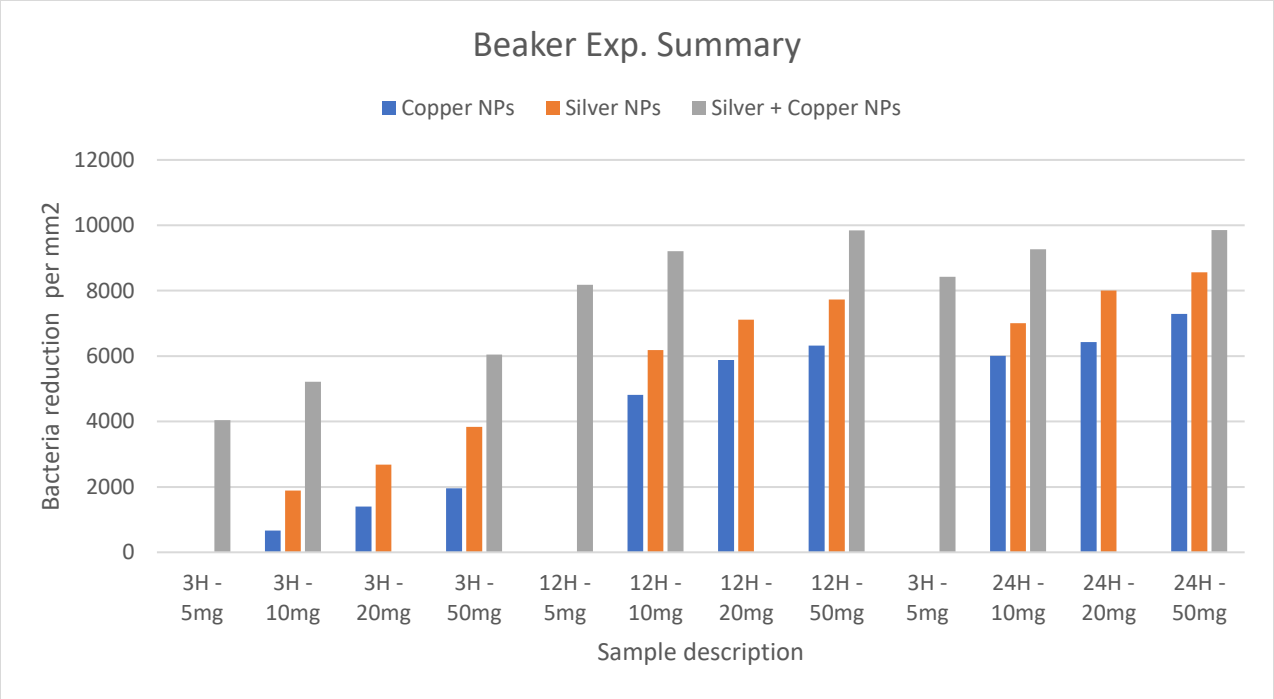

Figure 4.8: Beaker set-up summary

The copper nanoparticle treatment yielded the least reduction in all the concentrations and exposure periods with only 1 setup giving results above 7000per mm^2 bacterial count reduction. The silver nanoparticle experiment gave moderate results with 4 setups having reductions above 7000per mm^2 bacterial count. The copper and silver nanoparticle experiment proved synergetic effects of the chemicals as it resulted in 6 setups giving a reduction above 7000per mm^2 bacterial count. The exposure time and concentration needed to reach the 7000per mm^2 bacterial count for the combined experiment is significantly less. The 12hour 5mg concentration gives a reduction above 8000per mm^2 bacterial count.

From the data of the beaker experiment it can be deduced that the optimum concentration and exposure time needed to eradicate biofilm bacteria effectively would be a combination of both, silver and copper nanoparticles, with a concentration of 5mg per 25ml and exposure period of 9hours.

4.5 Channel Setup Experiment

4..5.1 Silver (Ag) nanoparticle Treatment

Samples were exposed to different concentrations of silver nanoparticles and different flow rate for 12hours. Figure 4.9 shows the reduction in bacterial count on the surfaces of these samples, obtained in comparison with the control samples taken out at the time of testing of each experiment.

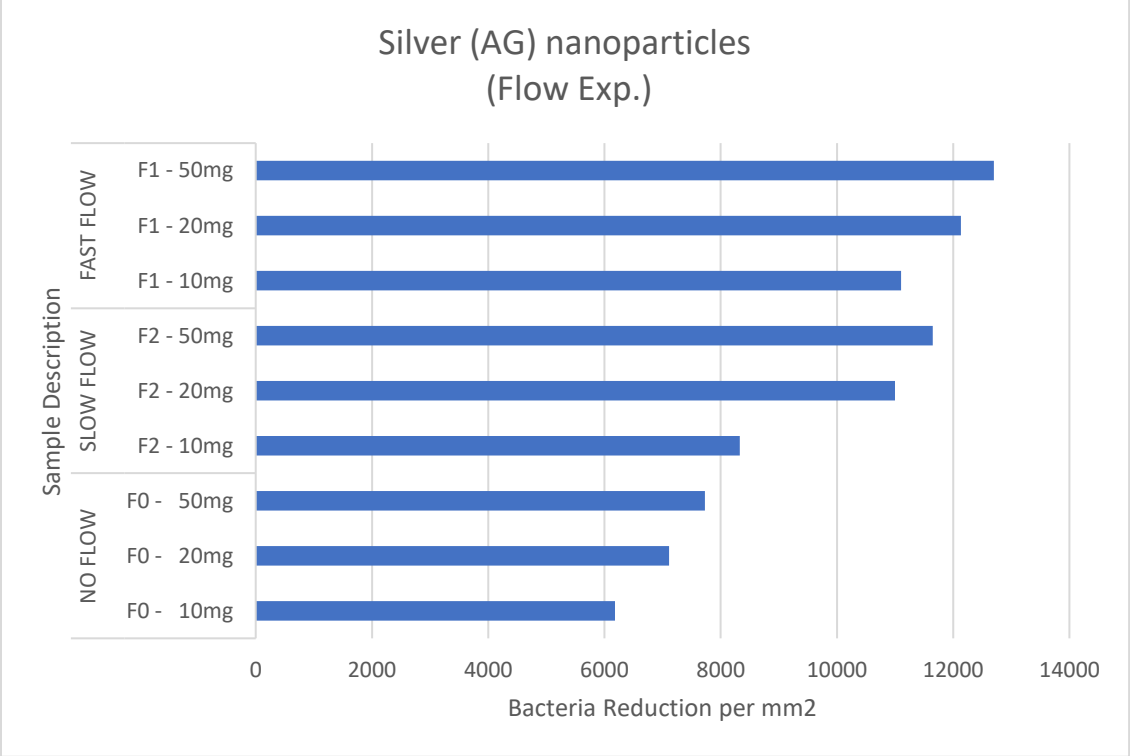

Figure 4.9: Channel set-up Silver nanoparticle treatment results

The flow rates of F1 (Flow 1) were higher than that of F2 (Flow2) and seen in Table 3.3. The effects of flow as expected, aided the reduction in the experiments represented above. All Flow1 experiments produced higher reductions than that of flow 2. In addition to this trend, the higher concentrations also gave larger reduction in the bacterial count on the sample surfaces. In comparison to the stationary treatment conditions in the beaker experiment, where the 10mg 12hour beaker experiment yielded about 6300per mm^2 bacterial count, the Flow2 10mg 12hour gave a reduction above 8000per mm^2 bacterial count.

Noted was a large amount of the nanoparticles on the surfaces of the experiment setup as seen in Figure A.2 (Appendix A).

4.5.2 Copper (Cu) nanoparticle Treatment

Samples were exposed to different concentrations of Copper nanoparticles and different flow rate for 12hours. Figure 4.10 shows the reduction in bacterial count on the surfaces of these samples, obtained in comparison with the control samples taken out at the time of testing of each experiment.

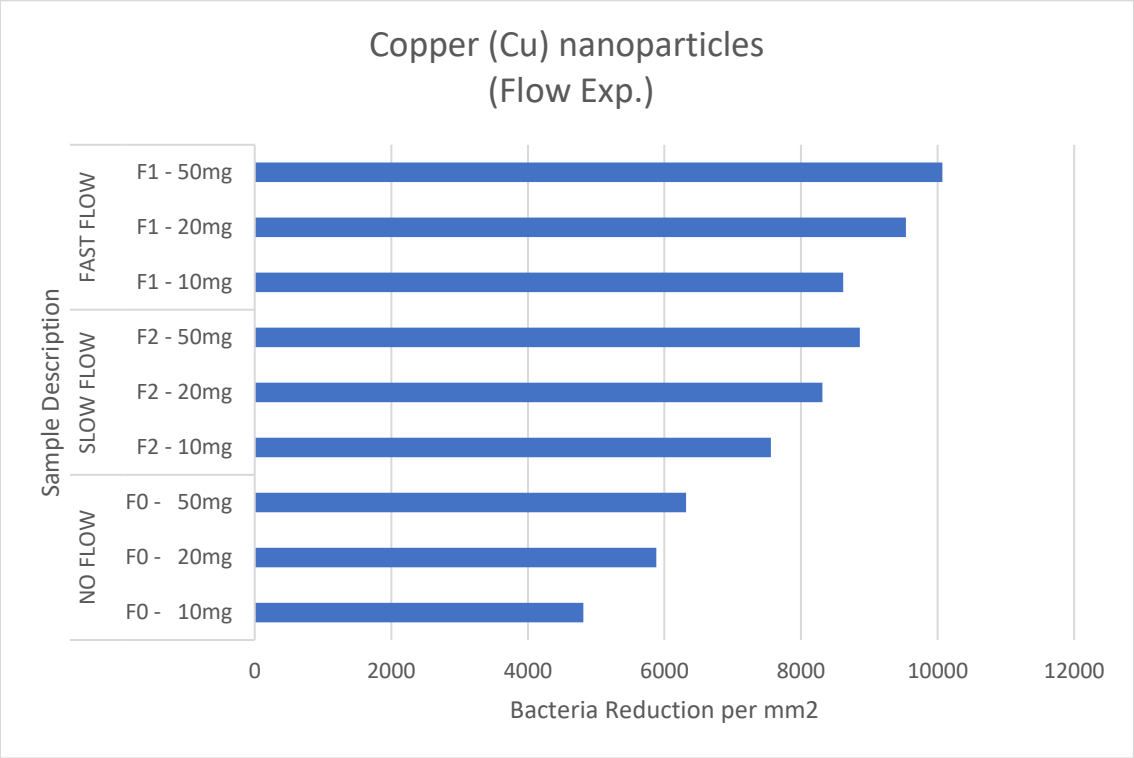

Figure 4.10: Channel set-up Copper nanoparticle treatment results

The general trend of the copper nanoparticle experiment is similar to that of the silver nanoparticle experiment in that the Flow1 experiments have a much greater reduction than that of the Flow2 experiments. This is because the flow rates of F1 (Flow 1) were higher than that of F2 (Flow2) and seen in Table 3.8. Flow can be regarded as a positive factor in the treatment of biofilm bacteria. The higher concentrations also yielded larger reductions. In comparison to the stationary

experimental results of the copper nanoparticle beaker experiment which had 4800per mm^2 bacterial count reduction, the channel Flow2 10mg 12hour experiment gave about 7600per mm^2 bacterial count reduction.

Noted was a large amount of the nanoparticles on the surfaces of the experiment setup as seen in Figure A.5 (Appendix A).

4.5.3 Combined Treatment of Silver and Copper (Ag+Cu) nanoparticles

Samples were exposed to different concentrations of combined Silver and Copper nanoparticles and different flow rate for 12hours. Figure 4.11 shows the reduction in bacterial count on the surfaces of these samples, obtained in comparison with the control samples taken out at the time of testing of each experiment.

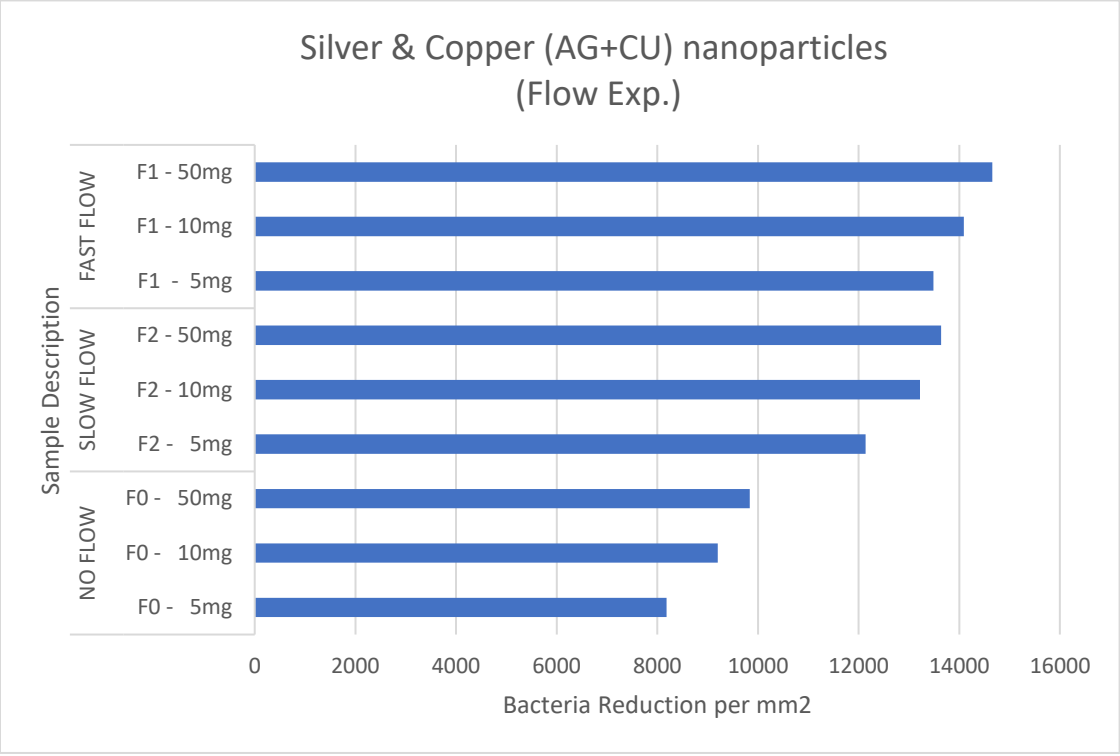

Figure 4.11: Channel set-up Silver and Copper nanoparticle treatment results

The differences Flow1 and Flow2 conditions were not as significant as in the individual chemical experiments due to the fact that the flows were not that much different as seen in Table 3.8. the

concentration trends remain the same as the higher concentrations have got larger concentration reductions. The Flow2 10mg 12hour channel experiment gave a 12000per mm^2 bacterial count reduction in comparison to the stationary beaker 10mg 12hour experiment which recorded a 9200per mm^2 bacterial count. This again proves that flow has a desired effect in the treatment of biofilm bacteria. This can be due to the shear effects that aid the chemicals to infiltrate the biofilm, or the chemical circulation helps in making sure that the complete surface area is contacted by the chemical.

4.6 Flow effects – Channel Experiment Summary

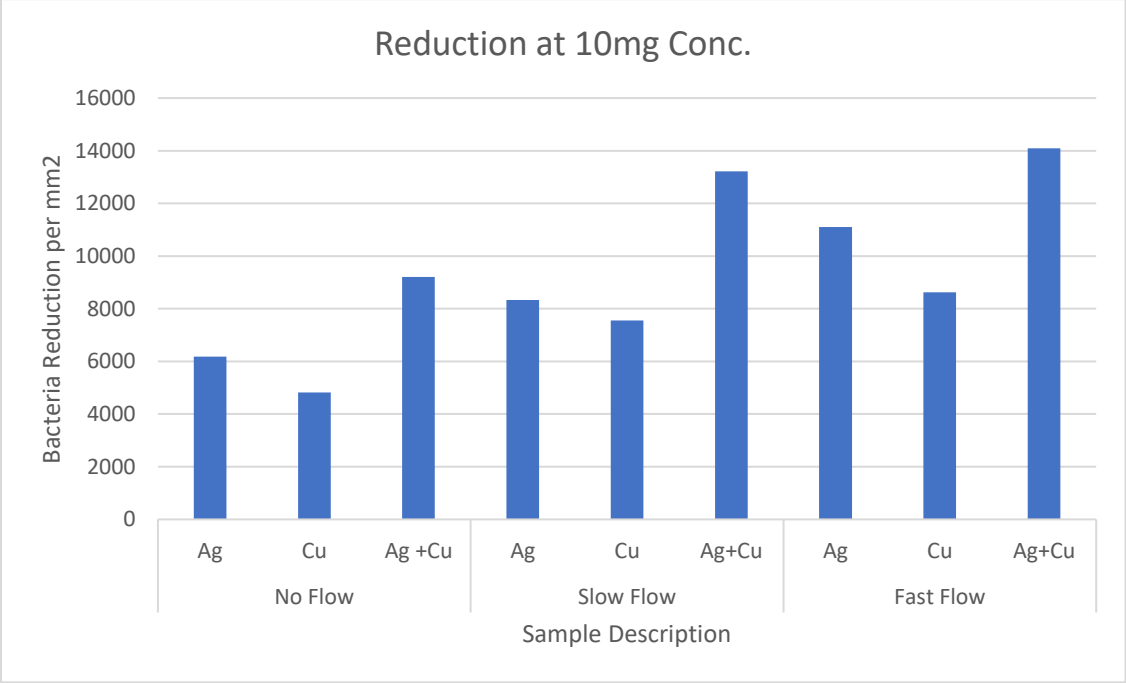

Figure 4.12: Bacterial reduction – effects of flow

Figure 4.12 displays the results at a fixed concentration (10mg per 25ml) and with a exposure time of 12H. this then displays the effects of flow of the reduction of bacteria. The results obtained show a marked increase in the bacterial reduction given a higher flow. The channel setup experiment results are combined below in Figure 4.13. The different flow rate effects can be examined over different concentrations and chemical combinations.

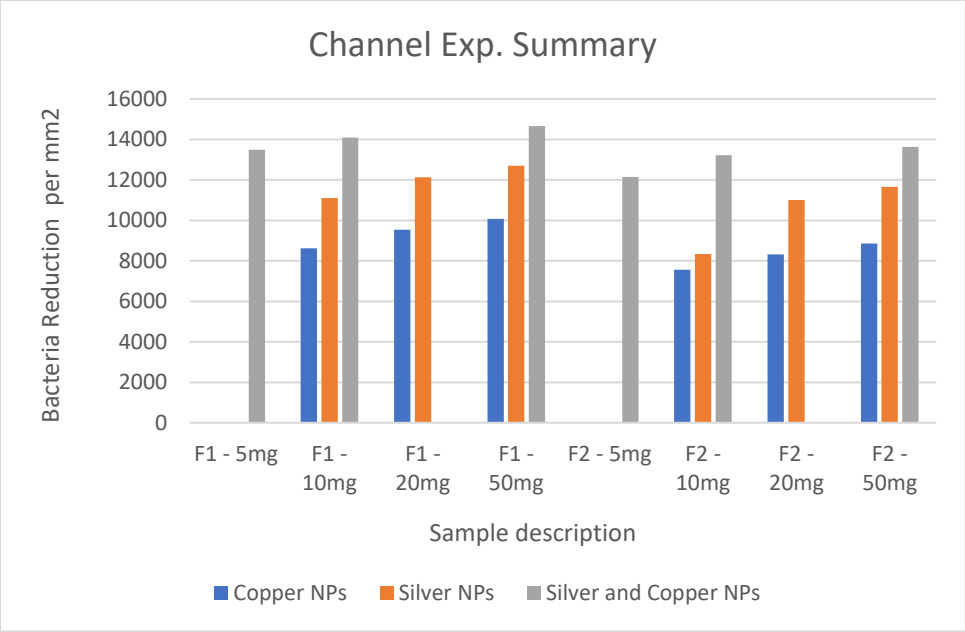

Figure 4.13: Channel set-up summary

Similar to the beaker experiment, the copper nanoparticle treated samples produced lower bacterial count reduction compared to the silver nanoparticle treated samples. The combined chemical treatment resulted in greater reductions in bacteria on the surface of the coupon samples. This proves the synergetic effects of the copper and silver nanoparticles in eradicating biofilm surface bacteria. The silver and copper nanoparticle combined 5mg Flow1 12hour experiment has very similar results to the 10mg Flow1 12hour experiment. This finding shows that when combined the concentrations needed to treat the bacteria are far less than that needed for the individual chemical treatment options.

4.7 Summary

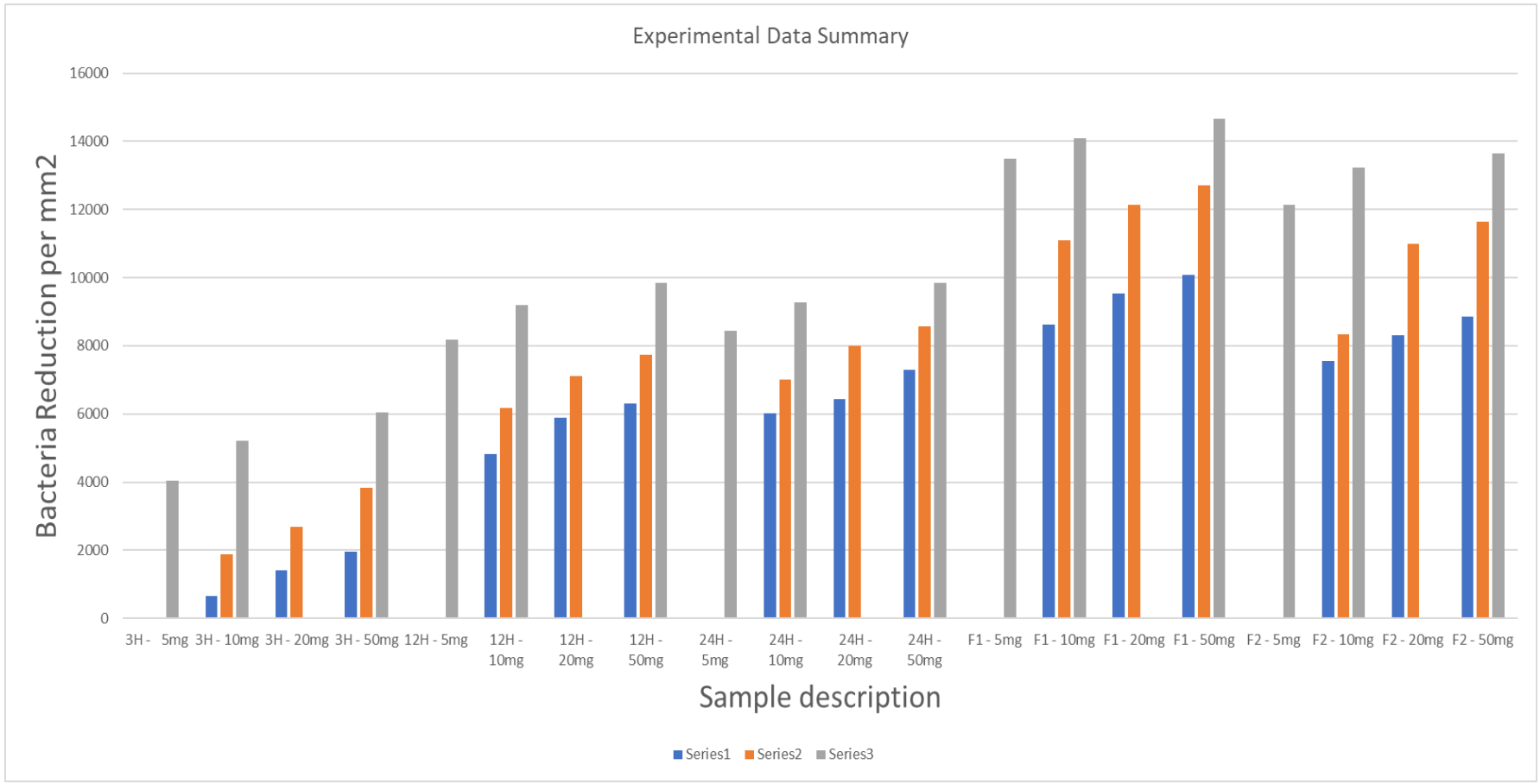

Figure 4.14 Results Summary

5. Concluding Remarks

The plausibility of treatment using these chemicals in industry heavily relies on the effective time of treatment and cost. Thus, finding the minimum concentration needed to achieve optimum reduction of biofilm bacteria in an acceptable time frame. This research aimed to investigate antibacterial effects of different copper and silver nanoparticle concentrations, with focus on determining the effective treatment time. In addition, the effects of flow and the synergetic effects of copper and silver nanoparticles when applied in combination.

Results displayed in Figure 4.14 summarise the all the experiments conducted. Observed are 2 general trends, firstly, the higher the concentrations gave higher reductions in biofilm bacteria count., secondly, the faster flows give larger reductions in biofilm bacteria count. The results support the expected desired synergetic effects of silver and copper nanoparticles. The combination of the two chemicals require far less concentration to be effective. Thus, its more cost effective and reduces health hazards to end users and limits pipe shut down time to a shorter period. The cost of synthesising silver nanoparticles is high; therefore it is an advantage to use the silver in combination with the copper because the silver is then required in smaller amounts. Use of other heavy metals could be investigated to achieve concentrations that are both harmless to the end consumer and that are cost effective to implement. The dislodged or detached dead bacteria from the treated biofilm will be present in the water post treatment, thus it is needed to investigate to what concentrations and the health impacts that the bacterial residue will have on the consumer and the environment.

The conducted experiment had limitations that include, the biofilm growth time, growth and treatment environmental conditions, bacterial count area percentage and chemical adhesion to apparatus surfaces. When studying the antibacterial effects of metal ions one should note when using 10^4 cells/mm and using 50ppb metals ions there will be about $2.8x10^{10}$ metal ions/ cell (Thurman, et al., 1989)The treated bacteria were grown over approximately 150 days. However, the biofilm in municipal pipelines have anything up to 10-year growth of biofilm. The difference in maturity of the biofilm may change the results obtained herein. Due to the vast number of samples the imagery of each sample was taken at less than 25% of the total area. Although random areas covering the entire sample were taken to circumvent the error due to this allot more a better method of imagery needs to be employed in future research. It was noted during the channel experiment and in reviewed literature, the issue of the nanoparticles adhering to the surfaces. This decreases the concentration of chemical in circulation. The results given are comparable with the literature findings and do show the syngenetic effects of the two chemicals employed.

The research gives confidence to warrant future investigations that will bring the technology closer to being implemented in industry. The issues of human health impacts need to be more thoroughly investigated in collaboration with health experts. In addition, the environmental impacts of having these heavy metals in the water would have needs to be assessed. An experiment with a fully operational pipeline is necessary in order to determine the cost and application procedure. From such an experiment a flushing out system can be obtained and all the environmental contingencies taken into consideration. The other future scope stemming from this research could be, testing the biofilm bacteria regrowth after chemical treatment, the lag time between application and when regrowth commences and thus the interval of treatment needed, the chemical concentrations in the actual flow, chemical application in closed channel flow. The above variables may affect the effects of the chemicals. One method to increase the interval of treatment is to make use of silver nanoparticles being embedded in matrices of the martial used to fabricate the pipe lining as done in food processing plants and some medical equipment. A quicker and more cost effective application of this research could be making a nanoparticle filter installed at the water-meters of each end user , or treating and flushing out the internal plumbing network of the end user.

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7. Appendix A

Herein are tables, equations and figures pertaining to the methodology aspect of the research above.

Table A.1: Flow rates - Silver and Copper nanoparticle channel experiment.

		T1	**T2**	**T3**	**AVE**	**Q(l/s)**
5mg AC	F1	91.43	90.4	91.5	91.1	0.007683
	F2	115.5	115.3	116.88	115.9	0.00604
10mg AC	F1	82.19	86.27	73.16	80.5	0.008691
	F2	188.17	193.64	192.35	191.4	0.003658
50mg AC	F1	53.42	69.38	51.38	58.1	0.012056
	F2	90.36	85.53	84.29	86.7	0.008071

Table A.2: Flow rates - Copper nanoparticle channel experiment.

		T1	**T2**	**T3**	**AVE**	**Q(l/s)**
10mg Cu	F1	48.00	47.74	50.16	48.6	0.014393
	F2	106.40	140.25	145.78	130.8	0.005351
20mg Cu	F1	53.16	49.85	50.90	51.3	0.013644
	F2	97.69	97.03	95.09	96.6	0.007246
50mg Cu	F1	79.56	80.88	75.90	78.8	0.008886
	F2	147.21	124.49	137.87	136.5	0.005127

Table A.3: Flow rates - Silver nanoparticle channel experiment.

		T1	T2	T3	AVE	Q(l/s)
10mg Ag	F1	33.53	33.07	33.04	33.2	0.021076
	F2	162.71	167.26	164.3	164.8	0.004249
20mg Ag	F1	49.61	46.94	47.78	48.1	0.01455
	F2	67.91	72.08	70.17	70.1	0.009992
50mg Ag	F1	32.49	32.12	32.75	32.5	0.021569
	F2	77.49	77.61	76.69	77.3	0.00906

Figure A.1: Channel setup experiment – Supply tanks (Silver nanoparticles)

Figure A.2: Channel Experiment – Nanoparticle sedimentation in supply tank

Figure A.3: Channel Experiment – Supply tank (Copper nanoparticles)

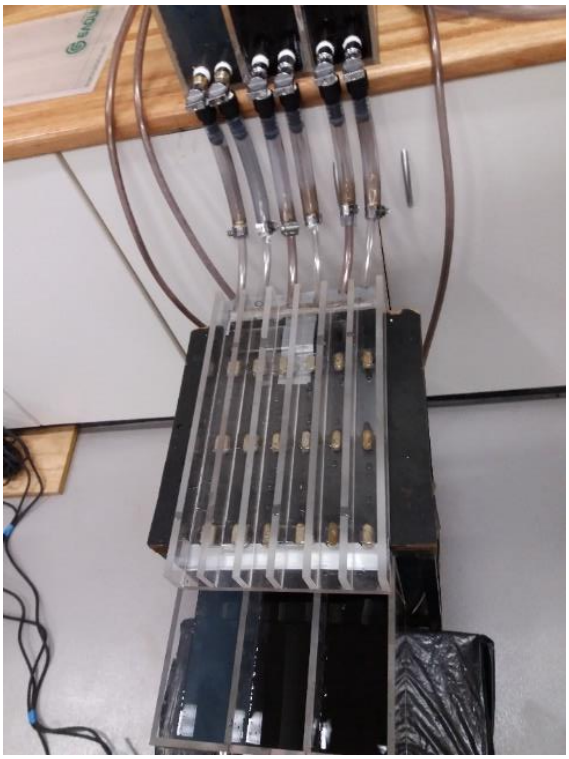

Figure A.4: Channel Experiment – Coupon layout

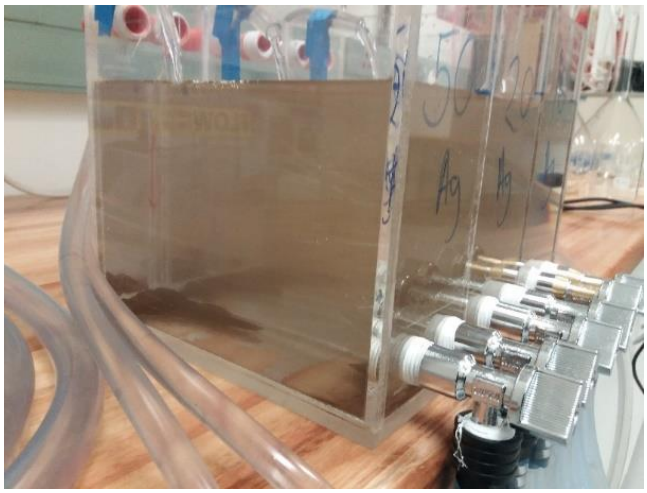

Figure A.5: Channel Experiment – Supply Tank Sedimentation

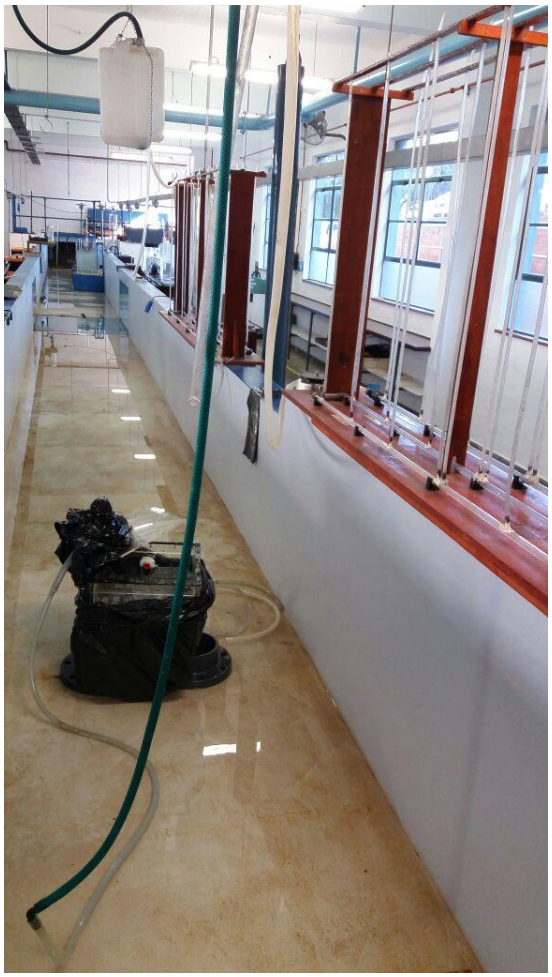

Figure A.6: Growth Tank

Figure A.7: Mortar Sample Coupon

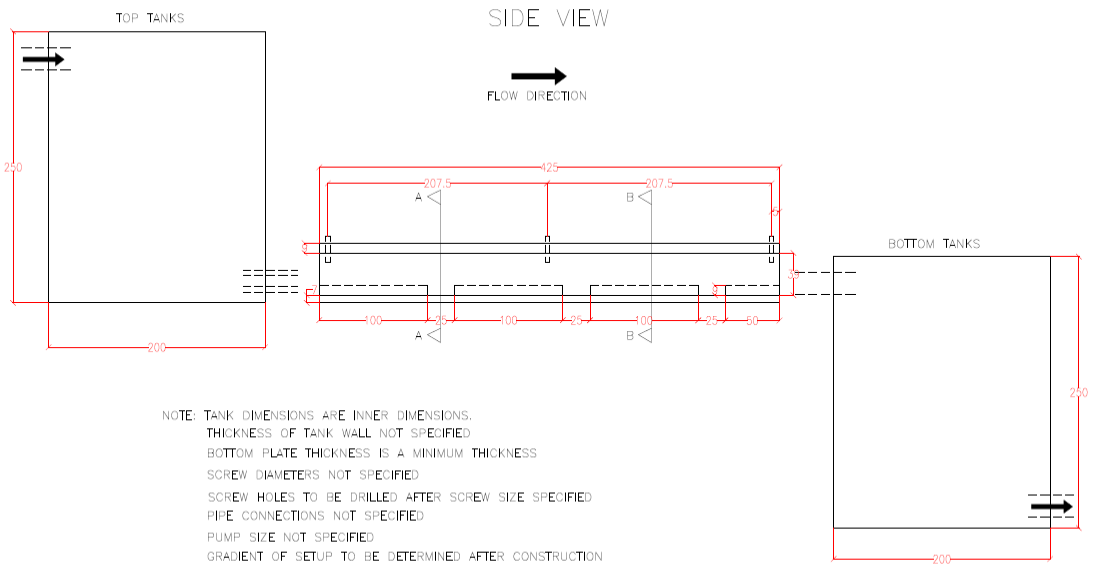

Figure A.8: Channel Experiment - Setup design (side view)

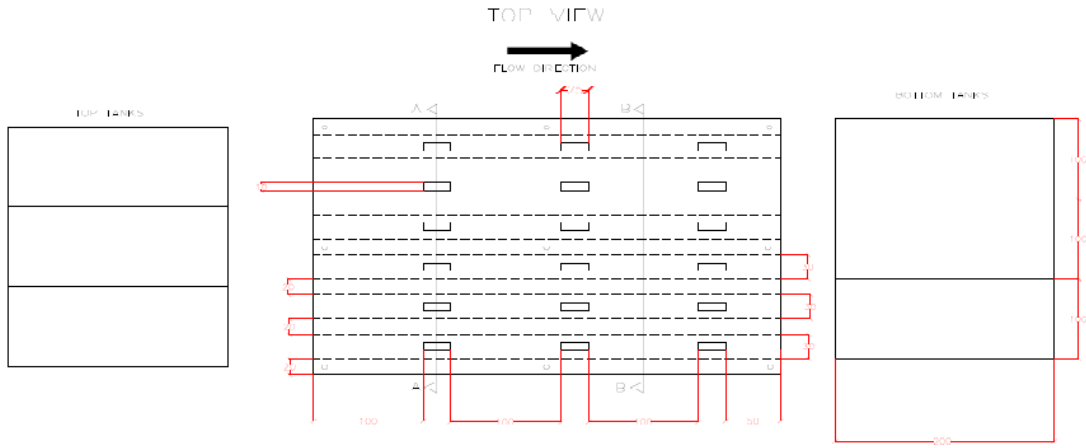

Figure A.9: Channel Experiment - Setup design (top view)

8. Appendix B

B.1 Results Tables

Table B.1.1: Results - Silver nanoparticle channel exp.

Silver (AG) nanoparticles - Channel Exp.					
F1 - 10mg	F1 - 20mg	F1 - 50mg	F2 - 10mg	F2 - 20mg	F2 - 50mg
11106	12134.49	12699.26	8329.473	11000.87	11648.9

Table B.1.2: Results - Copper nanoparticle channel exp.

Copper (Cu) nanoparticles - Channel Exp.					
F1 - 10mg	F1 - 20mg	F1 - 50mg	F2 - 10mg	F2 - 20mg	F2 - 50mg
8618.8	9538.258	10068.72	7556.853	8313.096	8862.464

Table B.1.3: Results – Silver and Copper nanoparticle channel exp.

Silver & Copper (AG+CU) nanoparticles - Channel Exp					
F1 - 10mg	F1 - 20mg	F1 - 50mg	F2 - 10mg	F2 - 20mg	F2 - 50mg
13486	14092.66	14656.04	12140.39	13220.02	13637.55

Table B.1.4: Results - Channel exp. Summary

Channel Exp. Summary								
	F1 - 5mg	F1 - 10mg	F1 - 20mg	F1 - 50mg	F2 - 5mg	F2 - 10mg	F2 - 20mg	F2 - 50mg
CU		8618.836	9538.258	10068.72		7556.853	8313.096	8862.464
AG		11105.67	12134.49	12699.26		8329.473	11000.87	11648.9
AC	13485.56	14092.66		14656.04	12140.39	13220.02		13637.55

Table B.1.5: Channel Exp. Results Data

Channel Exp. Results							
	F1			F2			CTRL
Ag	10	20	50	10	20	50	0
	16949	15920	15355	19725	17054	16406	28055
Cu	10	20	50	10	20	50	0
	20288	19368	18838	21350	20593	20044	28906
AC	5	10	50	5	10	50	0
	16439	15832	15269	17784	16705	16287	29925

Table B.1.6: Beaker Exp. Results Data

Beaker Exp. Results									
	3H			12H			24H		
Ag	10	20	50	10	20	50	10	20	50
	27181	26384	25238	25026	24095	23475	22067	21072	20511
Cu	10	20	50	10	20	50	10	20	50
	28402	27665	27107	26391	25323	24889	23065	22649	21788
AC	5	10	50	5	10	50	5	10	50
	25023	23857	23021	23023	22002	21366	20647	19805	19218
CTRL	0			0			0		
	29068			31206			29076		

Table B.1.7: Results - Silver nanoparticle beaker exp.

Silver (AG) nanoparticles - Beaker Exp								
3H - 10mg	3H - 20mg	3H - 50mg	12H - 10mg	12H - 20mg	12H - 50mg	24H - 10mg	24H - 20mg	24H - 50mg
1887	2685	3831	6180	7110	7731	7009	8004	8565

Table B.1.8: Results - Copper nanoparticle beaker exp.

Copper (CU) nanoparticles - Beaker Exp.								
3H - 10mg	3H - 20mg	3H - 50mg	12H - 10mg	12H - 20mg	12H - 50mg	24H - 10mg	24H - 20mg	24H - 50mg
667	1403	1961	4815	5883	6316	6011	6427	7288

Table B.1.9: Results – Silver and Copper nanoparticle beaker exp.

Silver & Copper (AG+CU) nanoparticles - Beaker Exp								
3H - 5mg	3H - 10mg	3H - 50mg	12H - 5mg	12H - 10mg	12H - 50mg	24H - 5mg	24H - 10mg	24H - 50mg
4045	5211	6048	8183	9203	9839	8429	9271	9858

B.2 Channel Experiment Data Readings from Images

For the purpose of the tables below the following are a guidline to the table titles given.

C – Channel experiment

B - Beaker experiment

Ag – Silver nanoparticles

Cu – Copper nanoparticles

AC – Silver and Copper nanoparticles

10mg - 10mg per 25ml concentration

20mg – 20mg per 25ml concentration

50mg - 50mg per 25ml concentration

3HR ,12HR, 24HR - Exposure period in hours

Table B.2.1: C-Ag-10mg-12HR-Flow1

C - Ag- 10mg - 12HR - Flow 1			
SAMPLE		NUMBER	CONC.
C Ag 10 12 F1 01	0.0553	741	13400
C Ag 10 12 F1 02	0.0192	356	18542
C Ag 10 12 F1 03	0.021	365	17381
C Ag 10 12 F1 04	0.0171	332	19415
C Ag 10 12 F1 05	0.0163	298	18282
C Ag 10 12 F1 06	0.0145	303	20897
C Ag 10 12 F1 07	0.0192	365	19010
C Ag 10 12 F1 08	0.0252	207	8214
C Ag 10 12 F1 09	0.0145	275	18966
C Ag 10 12 F1 10	0.0171	341	19942
C Ag 10 12 F1 11	0.0282	481	17057
C Ag 10 12 F1 12	0.0267	385	14419
C Ag 10 12 F1 13	0.0192	369	19219
C Ag 10 12 F1 14	0.0192	244	12708
C Ag 10 12 F1 15	0.0171	287	16784
AVE			16949
STD			3401

Table B.2.2: C-Ag-20mg-12HR-Flow1

C - Ag- 20mg - 12HR - Flow 1			
SAMPLE		NUMBER	CONC.
C Ag 20 12 F1 01	0.0145	227	15655.17
C Ag 20 12 F1 02	0.0171	295	17251.46
C Ag 20 12 F1 03	0.0282	350	12411.35
C Ag 20 12 F1 04	0.0267	314	11760.3
C Ag 20 12 F1 05	0.0192	207	10781.25
C Ag 20 12 F1 06	0.0145	263	18137.93
C Ag 20 12 F1 07	0.0171	336	19649.12
C Ag 20 12 F1 08	0.0253	412	16284.58
C Ag 20 12 F1 09	0.0192	357	18593.75
C Ag 20 12 F1 10	0.0192	325	16927.08
C Ag 20 12 F1 11	0.0192	355	18489.58
C Ag 20 12 F1 12	0.0192	369	19218.75
C Ag 20 12 F1 13	0.0256	337	13164.06
C Ag 20 12 F1 14	0.0252	452	17936.51
C Ag 20 12 F1 15	0.024	301	12541.67
AVE			15920.17
STD			2994.569

Table B.2.3: C-Ag-50mg-12HR-Flow1

C - Ag- 50mg - 12HR - Flow 1			
SAMPLE		NUMBER	CONC.
C Ag 50 12 F1 01	0.0176	258	14659.09
C Ag 50 12 F1 02	0.025	446	17840
C Ag 50 12 F1 03	0.057	688	12070.18
C Ag 50 12 F1 04	0.025	364	14560
C Ag 50 12 F1 05	0.0192	301	15677.08
C Ag 50 12 F1 06	0.04	357	8925
C Ag 50 12 F1 07	0.034	555	16323.53
C Ag 50 12 F1 08	0.0192	273	14218.75
C Ag 50 12 F1 09	0.025	432	17280
C Ag 50 12 F1 10	0.024	417	17375
C Ag 50 12 F1 11	0.022	416	18909.09
C Ag 50 12 F1 12	0.038	546	14368.42
C Ag 50 12 F1 13	0.0192	277	14427.08
C Ag 50 12 F1 14	0.0192	344	17916.67
C Ag 50 12 F1 15	0.0192	303	15781.25
AVE			15355.41
STD			2546.077

Table B.2.4: C-Ag-10mg-12HR-Flow2

C - Ag- 10mg - 12HR - Flow 2			
SAMPLE		NUMBER	CONC.
C Ag 10 12 F2 01	0.0192	199	10364.58
C Ag 10 12 F2 02	0.0192	336	17500
C Ag 10 12 F2 03	0.0384	741	19296.88
C Ag 10 12 F2 04	0.0252	479	19007.94
C Ag 10 12 F2 05	0.014	416	29714.29
C Ag 10 12 F2 06	0.0384	665	17317.71
C Ag 10 12 F2 07	0.0216	410	18981.48
C Ag 10 12 F2 08	0.0256	398	15546.88
C Ag 10 12 F2 09	0.0192	447	23281.25
C Ag 10 12 F2 10	0.0256	446	17421.88
C Ag 10 12 F2 11	0.0256	681	26601.56
C Ag 10 12 F2 12	0.0256	665	25976.56
C Ag 10 12 F2 13	0.0192	337	17552.08
C Ag 10 12 F2 14	0.0192	336	17500
C Ag 10 12 F2 15	0.0216	428	19814.81
AVE			19725.19
STD			4861.339

Table B.2.5: C-Ag-20mg-12HR-Flow2

C - Ag- 20mg - 12HR - Flow 2			
SAMPLE		NUMBER	CONC.
C Ag 20 12 F2 01	0.0192	412	21458.33
C Ag 20 12 F2 02	0.025	312	12480
C Ag 20 12 F2 03	0.0192	478	24895.83
C Ag 20 12 F2 04	0.04	336	8400
C Ag 20 12 F2 05	0.034	412	12117.65
C Ag 20 12 F2 06	0.0192	374	19479.17
C Ag 20 12 F2 07	0.025	341	13640
C Ag 20 12 F2 08	0.024	579	24125
C Ag 20 12 F2 09	0.0192	416	21666.67
C Ag 20 12 F2 10	0.0192	332	17291.67
C Ag 20 12 F2 11	0.0192	417	21718.75
C Ag 20 12 F2 12	0.0256	340	13281.25
C Ag 20 12 F2 13	0.0256	347	13554.69
C Ag 20 12 F2 14	0.0256	468	18281.25
C Ag 20 12 F2 15	0.024	322	13416.67
AVE			17053.79
STD			5020.795

Table B.2.6: C-Ag-50mg-12HR-Flow2

C - Ag- 50mg - 12HR - Flow 2			
SAMPLE		NUMBER	CONC.
C Ag 50 12 F2 01	0.0282	410	14539.01
C Ag 50 12 F2 02	0.0267	477	17865.17
C Ag 50 12 F2 03	0.0258	333	12906.98
C Ag 50 12 F2 04	0.0256	397	15507.81
C Ag 50 12 F2 05	0.0256	478	18671.88
C Ag 50 12 F2 06	0.0256	309	12070.31
C Ag 50 12 F2 07	0.0192	369	19218.75
C Ag 50 12 F2 08	0.04	596	14900
C Ag 50 12 F2 09	0.034	444	13058.82
C Ag 50 12 F2 10	0.0192	417	21718.75
C Ag 50 12 F2 11	0.0267	493	18464.42
C Ag 50 12 F2 12	0.0192	288	15000
C Ag 50 12 F2 13	0.0145	365	25172.41
C Ag 50 12 F2 14	0.0171	302	17660.82
C Ag 50 12 F2 15	0.0658	614	9331.307
AVE			16405.76
STD			4025.769

Table B.2.7: Control – C -Ag

Control - C - Ag			
SAMPLE		NUMBER	CONC.
Ctrl C Ag 01	0.0282	635	22517.7
Ctrl C Ag 02	0.0267	497	18614.2
Ctrl C Ag 03	0.0145	854	58896.6
Ctrl C Ag 04	0.036	924	25666.7
Ctrl C Ag 05	0.0253	665	26284.6
Ctrl C Ag 06	0.0192	887	46197.9
Ctrl C Ag 07	0.0192	552	28750
Ctrl C Ag 08	0.0192	547	28489.6
Ctrl C Ag 09	0.0192	608	31666.7
Ctrl C Ag 10	0.0192	479	24947.9
Ctrl C Ag 11	0.038	588	15473.7
Ctrl C Ag 12	0.0192	456	23750
Ctrl C Ag 13	0.0192	546	28437.5
Ctrl C Ag 14	0.0171	473	27660.8
Ctrl C Ag 15	0.0251	338	13466.1
AVE			28054.7
STD			11409

Table B.2.8: C-Cu-10mg-12HR-Flow1

C - Cu- 10mg - 12HR - Flow 1			
SAMPLE		NUMBER	CONC.
C Cu 10 12 F1 01	0.0145	288	19862.07
C Cu 10 12 F1 02	0.0171	352	20584.8
C Cu 10 12 F1 03	0.0282	436	15460.99
C Cu 10 12 F1 04	0.0267	471	17640.45
C Cu 10 12 F1 05	0.0145	367	25310.34
C Cu 10 12 F1 06	0.0171	365	21345.03
C Cu 10 12 F1 07	0.0282	471	16702.13
C Cu 10 12 F1 08	0.0267	488	18277.15
C Cu 10 12 F1 09	0.024	500	20833.33
C Cu 10 12 F1 10	0.022	481	21863.64
C Cu 10 12 F1 11	0.038	960	25263.16
C Cu 10 12 F1 12	0.0192	447	23281.25
C Cu 10 12 F1 13	0.0267	488	18277.15
C Cu 10 12 F1 14	0.0145	316	21793.1
C Cu 10 12 F1 15	0.0055	98	17818.18
AVE			20287.52
STD			2960.729

Table B.2.9: C-Cu-20mg-12HR-Flow1

C - Cu- 20mg - 12HR - Flow 1			
SAMPLE		NUMBER	CONC.
C Cu 20 12 F1 01	0.0253	325	12845.85
C Cu 20 12 F1 02	0.0192	409	21302.08
C Cu 20 12 F1 03	0.0192	355	18489.58
C Cu 20 12 F1 04	0.0192	389	20260.42
C Cu 20 12 F1 05	0.0192	358	18645.83
C Cu 20 12 F1 06	0.0256	471	18398.44
C Cu 20 12 F1 07	0.0282	367	13014.18
C Cu 20 12 F1 08	0.0267	502	18801.5
C Cu 20 12 F1 09	0.0325	607	18676.92
C Cu 20 12 F1 10	0.0154	359	23311.69
C Cu 20 12 F1 11	0.122	1266	10377.05
C Cu 20 12 F1 12	0.046	879	19108.7
C Cu 20 12 F1 13	0.0365	463	12684.93
C Cu 20 12 F1 14	0.0147	480	32653.06
C Ag 20 12 F1 15	0.0123	393	31951.22
AVE			19368.1
STD			6360.756

Table B.2.10: C-Cu-50mg-12HR-Flow1

C - Cu- 50mg - 12HR - Flow 1			
SAMPLE		NUMBER	CONC.
C Cu 50 12 F1 01	0.0256	471	18398.44
C Cu 50 12 F1 02	0.0282	367	13014.18
C Cu 50 12 F1 03	0.0267	502	18801.5
C Cu 50 12 F1 04	0.0325	607	18676.92
C Cu 50 12 F1 05	0.0154	205	13311.69
C Cu 50 12 F1 06	0.122	364	2983.607
C Cu 50 12 F1 07	0.046	301	6543.478
C Cu 50 12 F1 08	0.0365	357	9780.822
C Cu 50 12 F1 09	0.0147	555	37755.1
C Cu 50 12 F1 10	0.0123	273	22195.12
C Cu 50 12 F1 11	0.021	432	20571.43
C Cu 50 12 F1 12	0.0171	417	24385.96
C Cu 50 12 F1 13	0.0163	416	25521.47
C Cu 50 12 F1 14	0.0145	489	33724.14
C Cu 50 12 F1 15	0.0171	289	16900.58
AVE			18837.63
STD			9343.287

Table B.2.11: C-Cu-10mg-12HR-Flow2

C - Cu- 10mg - 12HR - Flow 2			
SAMPLE		NUMBER	CONC.
C Cu 10 12 F2 01	0.0282	557	19751.77
C Cu 10 12 F2 02	0.0267	781	29250.94
C Cu 10 12 F2 03	0.0325	784	24123.08
C Cu 10 12 F2 04	0.0192	363	18906.25
C Cu 10 12 F2 05	0.122	979	8024.59
C Cu 10 12 F2 06	0.0192	367	19114.58
C Cu 10 12 F2 07	0.0192	512	26666.67
C Cu 10 12 F2 08	0.024	479	19958.33
C Cu 10 12 F2 09	0.0192	241	12552.08
C Cu 10 12 F2 10	0.038	788	20736.84
C Cu 10 12 F2 11	0.0253	447	17667.98
C Cu 10 12 F2 12	0.0192	488	25416.67
C Cu 10 12 F2 13	0.021	611	29095.24
C Cu 10 12 F2 14	0.0171	399	23333.33
C Cu 10 12 F2 15	0.0163	418	25644.17
AVE			21349.5
STD			5865.424

Table B.2.12: C-Cu-20mg-12HR-Flow1

C - Cu- 20mg - 12HR - Flow 2			
SAMPLE		NUMBER	CONC.
C Cu 20 12 F2 01	0.046	705	15326.09
C Cu 20 12 F2 02	0.0365	579	15863.01
C Cu 20 12 F2 03	0.024	416	17333.33
C Cu 20 12 F2 04	0.0192	396	20625
C Cu 20 12 F2 05	0.0192	417	21718.75
C Cu 20 12 F2 06	0.0192	367	19114.58
C Cu 20 12 F2 07	0.0192	347	18072.92
C Cu 20 12 F2 08	0.04	667	16675
C Cu 20 12 F2 09	0.034	592	17411.76
C Cu 20 12 F2 10	0.0192	697	36302.08
C Cu 20 12 F2 11	0.0282	447	15851.06
C Cu 20 12 F2 12	0.0267	488	18277.15
C Cu 20 12 F2 13	0.0256	509	19882.81
C Cu 20 12 F2 14	0.0256	704	27500
C Cu 20 12 F2 15	0.0256	741	28945.31
AVE			20593.26
STD			5907.688

Table B.2.13: C-Cu-50mg-12HR-Flow2

C - Cu- 50mg - 12HR - Flow 2			
SAMPLE		NUMBER	CONC.
C Cu 50 12 F2 01	0.0282	410	14539.01
C Cu 50 12 F2 02	0.0267	398	14906.37
C Cu 50 12 F2 03	0.046	974	21173.91
C Cu 50 12 F2 04	0.0192	446	23229.17
C Cu 50 12 F2 05	0.04	668	16700
C Cu 50 12 F2 06	0.034	669	19676.47
C Cu 50 12 F2 07	0.0192	389	20260.42
C Cu 50 12 F2 08	0.0267	789	29550.56
C Cu 50 12 F2 09	0.0192	697	36302.08
C Cu 50 12 F2 10	0.0145	278	19172.41
C Cu 50 12 F2 11	0.0171	363	21228.07
C Cu 50 12 F2 12	0.046	979	21282.61
C Cu 50 12 F2 13	0.046	367	7978.261
C Cu 50 12 F2 14	0.0365	639	17506.85
C Cu 50 12 F2 15	0.046	789	17152.17
AVE			20043.89
STD			6537.06

Table B.2.14: Control – C - Cu

Control - C - Cu			
SAMPLE		NUMBER	CONC.
Ctrl C Cu 01	0.0282	987	35000
Ctrl C Cu 02	0.0267	497	18614.2
Ctrl C Cu 03	0.0145	854	58896.6
Ctrl C Cu 04	0.0267	887	29962.5
Ctrl C Cu 05	0.046	512	14456.5
Ctrl C Cu 06	0.0192	479	46197.9
Ctrl C Cu 07	0.04	588	13875
Ctrl C Cu 08	0.034	788	23176.5
Ctrl C Cu 09	0.0192	608	31666.7
Ctrl C Cu 10	0.0267	800	29962.5
Ctrl C Cu 11	0.038	665	17500
Ctrl C Cu 12	0.0267	887	33221
Ctrl C Cu 13	0.0256	555	21679.7
Ctrl C Cu 14	0.0256	840	32812.5
Ctrl C Cu 15	0.0251	667	26573.7
AVE			28906.4
STD			12072.8

Table B.2.15: C-AC-5mg-12HR-Flow1

C - AC- 5mg - 12HR - Flow 1			
SAMPLE		NUMBER	CONC.
C AC 10 12 F1 01	0.0282	478	16950.35
C AC 10 12 F1 02	0.0267	213	7977.528
C AC 10 12 F1 03	0.0145	266	18344.83
C AC 10 12 F1 04	0.036	715	19861.11
C AC 10 12 F1 05	0.0253	463	18300.4
C AC 10 12 F1 06	0.0192	197	10260.42
C AC 10 12 F1 07	0.0192	308	16041.67
C AC 10 12 F1 08	0.0192	206	10729.17
C AC 10 12 F1 09	0.0192	447	23281.25
C AC 10 12 F1 10	0.0192	207	10781.25
C AC 10 12 F1 11	0.038	547	14394.74
C AC 10 12 F1 12	0.0192	447	23281.25
C AC 10 12 F1 13	0.0192	368	19166.67
C AC 10 12 F1 14	0.0171	336	19649.12
C AC 10 12 F1 15	0.0251	441	17569.72
AVE			16439.3
STD			4709.006

Table B.2.16: C-AC-10mg-12HR-Flow1

C - AC- 10mg - 12HR - Flow 1			
SAMPLE		NUMBER	CONC.
C AC 20 12 F1 01	0.102	965	9460.784
C AC 20 12 F1 02	0.0253	447	17667.98
C AC 20 12 F1 03	0.0192	334	17395.83
C AC 20 12 F1 04	0.0192	285	14843.75
C AC 20 12 F1 05	0.0192	302	15729.17
C AC 20 12 F1 06	0.0192	333	17343.75
C AC 20 12 F1 07	0.0282	352	12482.27
C AC 20 12 F1 08	0.0267	452	16928.84
C AC 20 12 F1 09	0.0145	236	16275.86
C AC 20 12 F1 10	0.0192	204	10625
C AC 20 12 F1 11	0.04	285	7125
C AC 20 12 F1 12	0.0192	879	45781.25
C AC 20 12 F1 13	0.038	463	12184.21
C AC 20 12 F1 14	0.0789	480	6083.65
C AC 20 12 F1 15	0.036	632	17555.56
AVE			15832.19
STD			9155.372

Table B.2.17: C-AC-50mg-12HR-Flow1

C - AC- 50mg - 12HR - Flow 1			
SAMPLE		NUMBER	CONC.
C AC 50 12 F1 01	0.0192	299	15572.92
C AC 50 12 F1 02	0.0192	358	18645.83
C AC 50 12 F1 03	0.0171	284	16608.19
C AC 50 12 F1 04	0.0556	974	17517.99
C AC 50 12 F1 05	0.0192	274	14270.83
C AC 50 12 F1 06	0.038	607	15973.68
C AC 50 12 F1 07	0.0282	359	12730.5
C AC 50 12 F1 08	0.0267	471	17640.45
C AC 50 12 F1 09	0.0145	203	14000
C AC 50 12 F1 10	0.0253	487	19249.01
C AC 50 12 F1 11	0.0192	314	16354.17
C AC 50 12 F1 12	0.0192	259	13489.58
C AC 50 12 F1 13	0.0192	322	16770.83
C AC 50 12 F1 14	0.0192	217	11302.08
C AC 50 12 F1 15	0.032	285	8906.25
AVE			15268.82
STD			2830.683

Table B.2.18: C-AC-5mg-12HR-Flow2

C - AC- 5mg - 12HR - Flow 2			
SAMPLE		NUMBER	CONC.
C AC 10 12 F2 01	0.0192	297	15468.75
C AC 10 12 F2 02	0.038	697	18342.11
C AC 10 12 F2 03	0.0253	365	14426.88
C AC 10 12 F2 04	0.0192	410	21354.17
C AC 10 12 F2 05	0.0192	426	22187.5
C AC 10 12 F2 06	0.0192	370	19270.83
C AC 10 12 F2 07	0.0192	302	15729.17
C AC 10 12 F2 08	0.0192	378	19687.5
C AC 10 12 F2 09	0.0192	352	18333.33
C AC 10 12 F2 10	0.0171	257	15029.24
C AC 10 12 F2 11	0.0282	360	12765.96
C AC 10 12 F2 12	0.0267	412	15430.71
C AC 10 12 F2 13	0.0145	257	17724.14
C AC 10 12 F2 14	0.0192	368	19166.67
C AC 10 12 F2 15	0.04	874	21850
AVE			17784.46
STD			2880.876

Table B.2.19: C-AC-10mg-12HR-Flow2

C - AC- 10mg - 12HR - Flow 2			
SAMPLE		NUMBER	CONC.
C AC 20 12 F2 01	0.0364	558	15329.67
C AC 20 12 F2 02	0.0253	363	14347.83
C AC 20 12 F2 03	0.0192	207	10781.25
C AC 20 12 F2 04	0.0192	367	19114.58
C AC 20 12 F2 05	0.0192	333	17343.75
C AC 20 12 F2 06	0.0192	365	19010.42
C AC 20 12 F2 07	0.0192	363	18906.25
C AC 20 12 F2 08	0.038	403	10605.26
C AC 20 12 F2 09	0.0258	697	27015.5
C AC 20 12 F2 10	0.0192	365	19010.42
C AC 20 12 F2 11	0.0192	410	21354.17
C AC 20 12 F2 12	0.0171	308	18011.7
C AC 20 12 F2 13	0.0282	370	13120.57
C AC 20 12 F2 14	0.0267	302	11310.86
C AC 20 12 F2 15	0.0145	222	15310.34
AVE			16704.84
STD			4431.652

Table B.2.20: C-AC-50mg-12HR-Flow2

C - AC- 50mg - 12HR - Flow 2			
SAMPLE		NUMBER	CONC.
C AC 50 12 F2 01	0.0192	363	18906.25
C AC 50 12 F2 02	0.0192	403	20989.58
C AC 50 12 F2 03	0.038	697	18342.11
C AC 50 12 F2 04	0.0258	596	23100.78
C AC 50 12 F2 05	0.0192	410	21354.17
C AC 50 12 F2 06	0.04	273	6825
C AC 50 12 F2 07	0.034	432	12705.88
C AC 50 12 F2 08	0.0267	417	15617.98
C AC 50 12 F2 09	0.0192	399	20781.25
C AC 50 12 F2 10	0.0145	258	17793.1
C AC 50 12 F2 11	0.0171	374	21871.35
C AC 50 12 F2 12	0.122	887	7270.492
C AC 50 12 F2 13	0.046	569	12369.57
C AC 50 12 F2 14	0.0365	444	12164.38
C Cu 50 12 F2 15	0.0147	209	14217.69
AVE			16287.3
STD			5207.664

Table B.2.21: Control – C - AC

Control - AC - Ag			
SAMPLE		NUMBER	CONC.
Ctrl C AC 01	0.0282	887	31453.9
Ctrl C AC 02	0.0267	512	19176
Ctrl C AC 03	0.0267	474	17752.8
Ctrl C AC 04	0.0192	204	10625
Ctrl C AC 05	0.0145	441	30413.8
Ctrl C AC 06	0.0171	810	47368.4
Ctrl C AC 07	0.122	614	5032.79
Ctrl C AC 08	0.0192	666	34687.5
Ctrl C AC 09	0.0192	794	41354.2
Ctrl C AC 10	0.0171	665	38888.9
Ctrl C AC 11	0.0251	887	35338.6
Ctrl C AC 12	0.0192	555	28906.3
Ctrl C AC 13	0.0253	837	33083
Ctrl C AC 14	0.0192	774	40312.5
Ctrl C AC 15	0.0192	662	34479.2
AVE			29924.9
STD			11848.1

B.3 Beaker Experiment Data Readings from Images

C – Channel experiment

B - Beaker experiment

Ag – Silver nanoparticles

Cu – Copper nanoparticles

AC – Silver and Copper nanoparticles

10mg - 10mg per 25ml concentration

20mg – 20mg per 25ml concentration

50mg - 50mg per 25ml concentration

3HR ,12HR, 24HR - Exposure period in hours

Table B.3.1: B-Ag-10mg-3HR

B - Ag- 10mg - 3HR			
SAMPLE		NUMBER	CONC.
B Ag 10 3 01	0.0365	632	17315.07
B Ag 10 3 02	0.024	687	28625
B Ag 10 3 03	0.0192	551	28697.92
B Ag 10 3 04	0.0192	478	24895.83
B Ag 10 3 05	0.0171	456	26666.67
B Ag 10 3 06	0.0251	745	29681.27
B Ag 10 3 07	0.0145	304	20965.52
B Ag 10 3 08	0.036	984	27333.33
B Ag 10 3 09	0.0253	571	22569.17
B Ag 10 3 10	0.0192	698	36354.17
B Ag 10 3 11	0.0192	514	26770.83
B Ag 10 3 12	0.0192	567	29531.25
B Ag 10 3 13	0.0267	668	25018.73
B Ag 10 3 14	0.0192	471	24531.25
B Ag 10 3 15	0.0145	562	38758.62
AVE			27180.98
STD			5404.772

Table B.3.2: B-Ag-20mg-3HR

B - Ag- 20mg - 3HR			
SAMPLE		NUMBER	CONC.
B Ag 20 3 01	0.0145	354	24413.79
B Ag 20 3 02	0.036	641	17805.56
B Ag 20 3 03	0.0253	645	25494.07
B Ag 20 3 04	0.0192	639	33281.25
B Ag 20 3 05	0.0192	704	36666.67
B Ag 20 3 06	0.0192	574	29895.83
B Ag 20 3 07	0.0365	984	26958.9
B Ag 20 3 08	0.024	571	23791.67
B Ag 20 3 09	0.0192	603	31406.25
B Ag 20 3 10	0.0192	514	26770.83
B Ag 20 3 11	0.0171	354	20701.75
B Ag 20 3 12	0.0251	478	19043.82
B Ag 20 3 13	0.0267	654	24494.38
B Ag 20 3 14	0.0192	511	26614.58
B Ag 20 3 15	0.0145	412	28413.79
AVE			26383.54
STD			5142.505

Table B.3.3: B-Ag-50mg-3HR

B - Ag- 50mg - 3HR			
SAMPLE		NUMBER	CONC.
B Ag 50 3 01	0.054	987	18277.78
B Ag 50 3 02	0.02	574	28700
B Ag 50 3 03	0.0145	984	67862.07
B Ag 50 3 04	0.036	571	15861.11
B Ag 50 3 05	0.0253	603	23833.99
B Ag 50 3 06	0.0192	514	26770.83
B Ag 50 3 07	0.0192	354	18437.5
B Ag 50 3 08	0.0192	478	24895.83
B Ag 50 3 09	0.0192	456	23750
B Ag 50 3 10	0.0192	456	23750
B Ag 50 3 11	0.0171	345	20175.44
B Ag 50 3 12	0.0251	571	22749
B Ag 50 3 13	0.0365	665	18219.18
B Ag 50 3 14	0.024	654	27250
B Ag 50 3 15	0.03	541	18033.33
AVE			25237.74
STD			12413.48

Table B.3.4: B-Cu-10mg-3HR

B - Cu- 10mg - 3HR			
SAMPLE		NUMBER	CONC.
B Cu 10 3 01	0.0267	887	33220.97
B Cu 10 3 02	0.0192	687	35781.25
B Cu 10 3 03	0.0145	501	34551.72
B Cu 10 3 04	0.0192	645	33593.75
B Cu 10 3 05	0.0192	364	18958.33
B Cu 10 3 06	0.0171	412	24093.57
B Cu 10 3 07	0.0251	941	37490.04
B Cu 10 3 08	0.0145	689	47517.24
B Cu 10 3 09	0.036	571	15861.11
B Cu 10 3 10	0.0253	365	14426.88
B Cu 10 3 11	0.0192	456	23750
B Cu 10 3 12	0.0192	364	18958.33
B Cu 10 3 13	0.0192	412	21458.33
B Cu 10 3 14	0.0365	941	25780.82
B Cu 10 3 15	0.024	974	40583.33
AVE			28401.71
STD			9874.395

Table B.3.5: B-Cu-20mg-3HR

B - Cu- 20mg - 3HR			
SAMPLE		NUMBER	CONC.
B Cu 20 3 01	0.0267	874	32734.08
B Cu 20 3 02	0.0192	412	21458.33
B Cu 20 3 03	0.0145	456	31448.28
B Cu 20 3 04	0.02	689	34450
B Cu 20 3 05	0.0145	571	39379.31
B Cu 20 3 06	0.036	665	18472.22
B Cu 20 3 07	0.0253	369	14584.98
B Cu 20 3 08	0.0192	452	23541.67
B Cu 20 3 09	0.0192	369	19218.75
B Cu 20 3 10	0.0192	412	21458.33
B Cu 20 3 11	0.0192	741	38593.75
B Cu 20 3 12	0.0192	631	32864.58
B Cu 20 3 13	0.0171	369	21578.95
B Cu 20 3 14	0.0251	665	26494.02
B Cu 20 3 15	0.02	774	38700
AVE			27665.15
STD			8241.473

Table B.3.6: B-Cu-50mg-3HR

B - Cu- 50mg - 3HR			
SAMPLE		NUMBER	CONC.
B Cu 50 3 01	0.03	637	21233.33
B Cu 50 3 02	0.02	478	23900
B Cu 50 3 03	0.0145	650	44827.59
B Cu 50 3 04	0.036	987	27416.67
B Cu 50 3 05	0.0253	456	18023.72
B Cu 50 3 06	0.0192	456	23750
B Cu 50 3 07	0.0192	689	35885.42
B Cu 50 3 08	0.0192	571	29739.58
B Cu 50 3 09	0.0192	665	34635.42
B Cu 50 3 10	0.0192	369	19218.75
B Cu 50 3 11	0.0171	452	26432.75
B Cu 50 3 12	0.0251	574	22868.53
B Cu 50 3 13	0.0365	698	19123.29
B Cu 50 3 14	0.024	741	30875
B Ag 50 3 15	0.022	631	28681.82
AVE			27107.46
STD			7340.712

Table B.3.7: B-AC-5mg-3HR

B - AC- 5mg - 3HR			
SAMPLE		NUMBER	CONC.
B AC 10 3 01	0.0192	553	28802.08
B AC 10 3 02	0.0192	530	27604.17
B AC 10 3 03	0.0171	400	23391.81
B AC 10 3 04	0.0251	698	27808.76
B AC 10 3 05	0.02	469	23450
B AC 10 3 06	0.0145	354	24413.79
B AC 10 3 07	0.036	478	13277.78
B AC 10 3 08	0.0253	672	26561.26
B AC 10 3 09	0.0192	496	25833.33
B AC 10 3 10	0.0192	402	20937.5
B AC 10 3 11	0.0192	411	21406.25
B AC 10 3 12	0.03	852	28400
B AC 10 3 13	0.0267	666	24943.82
B AC 10 3 14	0.0192	578	30104.17
B AC 10 3 15	0.0145	412	28413.79
AVE			25023.24
STD			4256.777

Table B.3.8: B-AC-10mg-3HR

B - AC- 10mg - 3HR			
SAMPLE		NUMBER	CONC.
B AC 20 3 01	0.0267	599	22434.46
B AC 20 3 02	0.0192	666	34687.5
B AC 20 3 03	0.0145	351	24206.9
B AC 20 3 04	0.0192	540	28125
B AC 20 3 05	0.0192	652	33958.33
B AC 20 3 06	0.0171	369	21578.95
B AC 20 3 07	0.0251	469	18685.26
B AC 20 3 08	0.0235	654	27829.79
B AC 20 3 09	0.0145	478	32965.52
B AC 20 3 10	0.036	672	18666.67
B AC 20 3 11	0.0253	496	19604.74
B AC 20 3 12	0.0192	402	20937.5
B AC 20 3 13	0.0192	651	33906.25
B AC 20 3 14	0.0192	352	18333.33
B AC 20 3 15	0.188	365	1941.489
AVE			23857.45
STD			8601.145

Table B.3.9: B-AC-50mg-3HR

B - AC- 50mg - 3HR			
SAMPLE		NUMBER	CONC.
B AC 50 3 01	0.0267	354	13258.43
B AC 50 3 02	0.0192	541	28177.08
B AC 50 3 03	0.0145	366	25241.38
B AC 50 3 04	0.0192	509	26510.42
B AC 50 3 05	0.0192	412	21458.33
B AC 50 3 06	0.0171	540	31578.95
B AC 50 3 07	0.0251	652	25976.1
B AC 50 3 08	0.014	354	25285.71
B AC 50 3 09	0.0145	366	25241.38
B AC 50 3 10	0.036	654	18166.67
B AC 50 3 11	0.0253	478	18893.28
B AC 50 3 12	0.0192	412	21458.33
B AC 50 3 13	0.0192	496	25833.33
B AC 50 3 14	0.0192	444	23125
B AC 50 3 15	0.085	1284	15105.88
AVE			23020.68
STD			4979.134

Table B.3.10: Control – B – 3HR

Control - B - 3HR			
SAMPLE		NUMBER	CONC.
Ctrl B 3HR 01	0.0282	874	30992.91
Ctrl B 3HR 02	0.0267	748	28014.98
Ctrl B 3HR 03	0.0145	887	61172.41
Ctrl B 3HR 04	0.036	687	19083.33
Ctrl B 3HR 05	0.0253	501	19802.37
Ctrl B 3HR 06	0.0192	645	15468.75
Ctrl B 3HR 07	0.0192	364	33593.75
Ctrl B 3HR 08	0.0192	412	23281.25
Ctrl B 3HR 09	0.0192	941	49010.42
Ctrl B 3HR 10	0.0192	689	35885.42
Ctrl B 3HR 11	0.038	571	15026.32
Ctrl B 3HR 12	0.0192	647	33697.92
Ctrl B 3HR 13	0.0192	297	15468.75
Ctrl B 3HR 14	0.0171	645	37719.3
Ctrl B 3HR 15	0.0251	447	17808.76
AVE			29068.44
STD			13387.89

Table B.3.11: B-Ag-10mg-12HR

B - Ag- 10mg - 12HR			
SAMPLE		NUMBER	CONC.
B Ag 10 12 01	0.02	354	17700
B Ag 10 12 02	0.0365	699	19150.68
B Ag 10 12 03	0.024	513	21375
B Ag 10 12 04	0.0192	441	22968.75
B Ag 10 12 05	0.0192	403	20989.58
B Ag 10 12 06	0.0171	704	41169.59
B Ag 10 12 07	0.0251	513	20438.25
B Ag 10 12 08	0.03	784	26133.33
B Ag 10 12 09	0.0145	654	45103.45
B Ag 10 12 10	0.036	405	11250
B Ag 10 12 11	0.0253	669	26442.69
B Ag 10 12 12	0.0192	651	33906.25
B Ag 10 12 13	0.0192	450	23437.5
B Ag 10 12 14	0.0192	441	22968.75
B Ag 10 12 15	0.02	447	22350
AVE			25025.59
STD			8834.179

Table B.3.12: B-Ag-20mg-12HR

B - Ag- 20mg - 12HR			
SAMPLE		NUMBER	CONC.
B Ag 20 12 01	0.0267	698	26142.32
B Ag 20 12 02	0.0192	441	22968.75
B Ag 20 12 03	0.0145	403	27793.1
B Ag 20 12 04	0.0365	724	19835.62
B Ag 20 12 05	0.024	596	24833.33
B Ag 20 12 06	0.0145	465	32068.97
B Ag 20 12 07	0.036	684	19000
B Ag 20 12 08	0.0253	456	18023.72
B Ag 20 12 09	0.0192	523	27239.58
B Ag 20 12 10	0.0192	478	24895.83
B Ag 20 12 11	0.0192	669	34843.75
B Ag 20 12 12	0.02	651	32550
B Ag 20 12 13	0.0365	658	18027.4
B Ag 20 12 14	0.024	441	18375
B Ag 20 12 15	0.03	445	14833.33
AVE			24095.38
STD			6077.43

Table B.3.13: B-Ag-50mg-12HR

B - Ag- 50mg - 12HR			
SAMPLE		NUMBER	CONC.
B Ag 50 12 01	0.0267	465	17415.73
B Ag 50 12 02	0.0192	331	17239.58
B Ag 50 12 03	0.0145	456	31448.28
B Ag 50 12 04	0.0192	523	27239.58
B Ag 50 12 05	0.0192	478	24895.83
B Ag 50 12 06	0.0171	396	23157.89
B Ag 50 12 07	0.0251	651	25936.25
B Ag 50 12 08	0.036	636	17666.67
B Ag 50 12 09	0.0253	789	31185.77
B Ag 50 12 10	0.0192	475	24739.58
B Ag 50 12 11	0.0192	455	23697.92
B Ag 50 12 12	0.0192	444	23125
B Ag 50 12 13	0.0365	697	19095.89
B Ag 50 12 14	0.024	513	21375
B Ag 50 12 15	0.033	789	23909.09
AVE			23475.2
STD			4474.185

Table B.3.14: B-Cu-10mg-12HR

B - Cu- 10mg - 12HR			
SAMPLE		NUMBER	CONC.
B Cu 10 12 01	0.036	665	18472.22
B Cu 10 12 02	0.0365	668	18301.37
B Cu 10 12 03	0.024	471	19625
B Cu 10 12 04	0.0145	661	45586.21
B Cu 10 12 05	0.036	674	18722.22
B Cu 10 12 06	0.0253	774	30592.89
B Cu 10 12 07	0.0192	513	26718.75
B Cu 10 12 08	0.0192	715	37239.58
B Cu 10 12 09	0.0192	654	34062.5
B Cu 10 12 10	0.0192	559	29114.58
B Cu 10 12 11	0.0192	452	23541.67
B Cu 10 12 12	0.0171	321	18771.93
B Cu 10 12 13	0.0251	651	25936.25
B Cu 10 12 14	0.0365	668	18301.37
B Cu 10 12 15	0.024	741	30875
AVE			26390.77
STD			8258.515

Table B.3.15: B-Cu-20mg-12HR

B - Cu- 20mg - 12HR			
SAMPLE		NUMBER	CONC.
B Cu 20 12 01	0.0267	462	17303.37
B Cu 20 12 02	0.0192	670	34895.83
B Cu 20 12 03	0.0145	354	24413.79
B Cu 20 12 04	0.0145	699	48206.9
B Cu 20 12 05	0.036	513	14250
B Cu 20 12 06	0.0253	715	28260.87
B Cu 20 12 07	0.0192	896	46666.67
B Cu 20 12 08	0.0192	559	29114.58
B Cu 20 12 09	0.0192	690	35937.5
B Cu 20 12 10	0.52	698	1342.308
B Cu 20 12 11	0.0365	654	17917.81
B Cu 20 12 12	0.024	565	23541.67
B Cu 20 12 13	0.0282	887	31453.9
B Cu 20 12 14	0.0267	684	25617.98
B Ag 20 12 15	0.85	780	917.6471
AVE			25322.72
STD			13785.79

Table B.3.16: B-Cu-50mg-12HR

B - Cu- 50mg - 12HR			
SAMPLE		NUMBER	CONC.
B Cu 50 12 01	0.0145	531	36620.69
B Cu 50 12 02	0.036	781	21694.44
B Cu 50 12 03	0.0253	896	35415.02
B Cu 50 12 04	0.0192	559	29114.58
B Cu 50 12 05	0.0192	558	29062.5
B Cu 50 12 06	0.0192	444	23125
B Cu 50 12 07	0.0365	697	19095.89
B Cu 50 12 08	0.024	412	17166.67
B Cu 50 12 09	0.0192	465	24218.75
B Cu 50 12 10	0.0192	405	21093.75
B Cu 50 12 11	0.0171	321	18771.93
B Cu 50 12 12	0.0251	651	25936.25
B Cu 50 12 13	0.0267	678	25393.26
B Cu 50 12 14	0.0192	412	21458.33
B Cu 50 12 15	0.0145	365	25172.41
AVE			24889.3
STD			5705.181

Table B.3.17: B-AC-5mg-12HR

B - AC- 5mg - 12HR			
SAMPLE		NUMBER	CONC.
B AC 10 12 01	0.0365	801	21945.21
B AC 10 12 02	0.024	603	25125
B AC 10 12 03	0.0145	336	23172.41
B AC 10 12 04	0.036	788	21888.89
B AC 10 12 05	0.0253	559	22094.86
B AC 10 12 06	0.0192	452	23541.67
B AC 10 12 07	0.0192	465	24218.75
B AC 10 12 08	0.0192	404	21041.67
B AC 10 12 09	0.0192	458	23854.17
B AC 10 12 10	0.0192	465	24218.75
B AC 10 12 11	0.0171	401	23450.29
B AC 10 12 12	0.0251	654	26055.78
B AC 10 12 13	0.0267	565	21161.05
B AC 10 12 14	0.0192	352	18333.33
B AC 10 12 15	0.0145	366	25241.38
AVE			23022.88
STD			1986.68

Table B.3.18: B-AC-10mg-12HR

B - AC- 10mg - 12HR			
SAMPLE		NUMBER	CONC.
B AC 20 12 01	0.074	1444	19513.51
B AC 20 12 02	0.0267	559	20936.33
B AC 20 12 03	0.0192	558	29062.5
B AC 20 12 04	0.0145	444	30620.69
B AC 20 12 05	0.024	559	23291.67
B AC 20 12 06	0.0365	690	18904.11
B AC 20 12 07	0.024	556	23166.67
B AC 20 12 08	0.362	654	1806.63
B AC 20 12 09	0.0145	412	28413.79
B AC 20 12 10	0.036	655	18194.44
B AC 20 12 11	0.0253	658	26007.91
B AC 20 12 12	0.0192	559	29114.58
B AC 20 12 13	0.0192	399	20781.25
B AC 20 12 14	0.0192	444	23125
B AC 20 12 15	0.074	1265	17094.59
AVE			22002.25
STD			7076.162

Table B.3.19: B-AC-50mg-12HR

B - AC- 50mg - 12HR			
SAMPLE		NUMBER	CONC.
B AC 50 12 01	0.0365	799	21890.41
B AC 50 12 02	0.0145	345	23793.1
B AC 50 12 03	0.036	558	15500
B AC 50 12 04	0.0253	444	17549.41
B AC 50 12 05	0.0192	559	29114.58
B AC 50 12 06	0.0192	360	18750
B AC 50 12 07	0.0192	369	19218.75
B AC 50 12 08	0.0241	654	27136.93
B AC 50 12 09	0.0192	368	19166.67
B AC 50 12 10	0.0192	401	20885.42
B AC 50 12 11	0.0171	411	24035.09
B AC 50 12 12	0.0251	558	22231.08
B AC 50 12 13	0.0267	444	16629.21
B AC 50 12 14	0.0192	451	23489.58
B AC 50 12 15	0.0145	306	21103.45
AVE			21366.25
STD			3778.667

Table B.3.20: Control – B – 12HR

Control - B - 12HR			
SAMPLE		NUMBER	CONC.
Ctrl B 12HR 01	0.0282	667	23652.48
Ctrl B 12HR 02	0.0267	471	17640.45
Ctrl B 12HR 03	0.0145	661	45586.21
Ctrl B 12HR 04	0.036	674	18722.22
Ctrl B 12HR 05	0.0253	774	30592.89
Ctrl B 12HR 06	0.0192	513	26718.75
Ctrl B 12HR 07	0.0192	715	37239.58
Ctrl B 12HR 08	0.0192	654	34062.5
Ctrl B 12HR 09	0.0192	779	40572.92
Ctrl B 12HR 10	0.0192	910	47395.83
Ctrl B 12HR 11	0.038	804	21157.89
Ctrl B 12HR 12	0.0192	704	36666.67
Ctrl B 12HR 13	0.0192	654	34062.5
Ctrl B 12HR 14	0.0171	551	32222.22
Ctrl B 12HR 15	0.0251	547	21792.83
AVE			31205.73
STD			9438.61

Table B.3.21: B-Ag-10mg-24HR

B - Ag- 10mg - 24HR			
SAMPLE		NUMBER	CONC.
B Ag 10 24 01	0.0192	361	18802.08
B Ag 10 24 02	0.0192	345	17968.75
B Ag 10 24 03	0.0171	402	23508.77
B Ag 10 24 04	0.0251	478	19043.82
B Ag 10 24 05	0.0145	303	20896.55
B Ag 10 24 06	0.036	651	18083.33
B Ag 10 24 07	0.0253	613	24229.25
B Ag 10 24 08	0.0192	405	21093.75
B Ag 10 24 09	0.0192	475	24739.58
B Ag 10 24 10	0.0192	441	22968.75
B Ag 10 24 11	0.0267	403	15093.63
B Ag 10 24 12	0.0192	724	37708.33
B Ag 10 24 13	0.0145	333	22965.52
B Ag 10 24 14	0.0365	562	15397.26
B Ag 10 24 15	0.024	684	28500
AVE			22066.63
STD			5665.982

Table B.3.22: B-Ag-20mg-24HR

B - Ag- 20mg - 24HR			
SAMPLE		NUMBER	CONC.
B Ag 20 24 01	0.0145	332	22896.55
B Ag 20 24 02	0.036	806	22388.89
B Ag 20 24 03	0.0253	455	17984.19
B Ag 20 24 04	0.0192	444	23125
B Ag 20 24 05	0.0192	364	18958.33
B Ag 20 24 06	0.0192	513	26718.75
B Ag 20 24 07	0.014	448	32000
B Ag 20 24 08	0.0365	669	18328.77
B Ag 20 24 09	0.024	406	16916.67
B Ag 20 24 10	0.074	999	13500
B Ag 20 24 11	0.0282	441	15638.3
B Ag 20 24 12	0.0267	654	24494.38
B Ag 20 24 13	0.0267	565	21161.05
B Ag 20 24 14	0.0192	410	21354.17
B Ag 20 24 15	0.0145	299	20620.69
AVE			21072.38
STD			4615.529

Table B.3.23: B-Ag-50mg-24HR

B - Ag- 50mg - 24HR			
SAMPLE		NUMBER	CONC.
B Ag 50 24 01	0.0267	566	21198.5
B Ag 50 24 02	0.0192	368	19166.67
B Ag 50 24 03	0.0145	400	27586.21
B Ag 50 24 04	0.0365	658	18027.4
B Ag 50 24 05	0.024	441	18375
B Ag 50 24 06	0.0282	354	12553.19
B Ag 50 24 07	0.0267	422	15805.24
B Ag 50 24 08	0.0282	513	18191.49
B Ag 50 24 09	0.0267	715	26779.03
B Ag 50 24 10	0.0192	401	20885.42
B Ag 50 24 11	0.0192	559	29114.58
B Ag 50 24 12	0.0192	654	34062.5
B Ag 50 24 13	0.0365	565	15479.45
B Ag 50 24 14	0.098	1203	12275.51
B Ag 50 24 15	0.036	654	18166.67
AVE			20511.12
STD			6265.831

Table B.3.24: B-Cu-10mg-24HR

B - Cu- 10mg - 24HR			
SAMPLE		NUMBER	CONC.
B Cu 10 24 01	0.0267	661	24756.55
B Cu 10 24 02	0.0192	677	35260.42
B Cu 10 24 03	0.0145	366	25241.38
B Cu 10 24 04	0.0192	513	26718.75
B Cu 10 24 05	0.0192	452	23541.67
B Cu 10 24 06	0.0171	502	29356.73
B Cu 10 24 07	0.0251	559	22270.92
B Cu 10 24 08	0.052	669	12865.38
B Cu 10 24 09	0.0145	364	25103.45
B Cu 10 24 10	0.036	587	16305.56
B Cu 10 24 11	0.0253	406	16047.43
B Cu 10 24 12	0.0192	499	25989.58
B Cu 10 24 13	0.0192	446	23229.17
B Cu 10 24 14	0.0192	441	22968.75
B Cu 10 24 15	0.034	555	16323.53
AVE			23065.28
STD			5777.297

Table B.3.25: B-Cu-20mg-24HR

B - Cu- 20mg - 24HR			
SAMPLE		NUMBER	CONC.
B Cu 20 24 01	0.024	599	24958.33
B Cu 20 24 02	0.0267	565	21161.05
B Cu 20 24 03	0.0192	402	20937.5
B Cu 20 24 04	0.0145	330	22758.62
B Cu 20 24 05	0.0282	669	23723.4
B Cu 20 24 06	0.0267	468	17528.09
B Cu 20 24 07	0.0282	658	23333.33
B Cu 20 24 08	0.0267	441	16516.85
B Cu 20 24 09	0.0365	687	18821.92
B Cu 20 24 10	0.024	699	29125
B Cu 20 24 11	0.0192	513	26718.75
B Cu 20 24 12	0.0192	715	37239.58
B Cu 20 24 13	0.0192	587	30572.92
B Cu 20 24 14	0.0365	559	15315.07
B Cu 20 24 15	0.04	441	11025
AVE			22649.03
STD			6620.657

Table B.3.26: B-AC-5mg-24HR

B - AC- 5mg - 24HR			
SAMPLE		NUMBER	CONC.
B AC 10 24 01	0.0145	444	30620.69
B AC 10 24 02	0.036	659	18305.56
B AC 10 24 03	0.0253	570	22529.64
B AC 10 24 04	0.0192	369	19218.75
B AC 10 24 05	0.0192	503	26197.92
B AC 10 24 06	0.0192	368	19166.67
B AC 10 24 07	0.0365	547	14986.3
B AC 10 24 08	0.024	478	19916.67
B AC 10 24 09	0.0192	320	16666.67
B AC 10 24 10	0.0192	394	20520.83
B AC 10 24 11	0.0171	325	19005.85
B AC 10 24 12	0.0251	536	21354.58
B AC 10 24 13	0.0267	658	24644.19
B AC 10 24 14	0.0192	334	17395.83
B AC 10 24 15	0.0145	278	19172.41
AVE			20646.84
STD			3999.081

Table B.3.27: B-Cu-50mg-24HR

B - Cu- 50mg - 24HR			
SAMPLE		NUMBER	CONC.
B Cu 50 24 01	0.0267	574	21498.13
B Cu 50 24 02	0.0192	354	18437.5
B Cu 50 24 03	0.0145	288	19862.07
B Cu 50 24 04	0.036	654	18166.67
B Cu 50 24 05	0.0365	715	19589.04
B Cu 50 24 06	0.024	674	28083.33
B Cu 50 24 07	0.0365	559	15315.07
B Cu 50 24 08	0.024	654	27250
B Cu 50 24 09	0.0282	565	20035.46
B Cu 50 24 10	0.0267	620	23220.97
B Cu 50 24 11	0.0365	478	13095.89
B Cu 50 24 12	0.024	669	27875
B Cu 50 24 13	0.0267	651	24382.02
B Cu 50 24 14	0.0365	764	20931.51
B Cu 50 24 15	0.024	698	29083.33
AVE			21788.4
STD			4801.381

Table B.3.28: B-AC-10mg-24HR

B - AC- 10mg - 24HR			
SAMPLE		NUMBER	CONC.
B AC 20 24 01	0.0282	690	24468.09
B AC 20 24 02	0.0267	556	20823.97
B AC 20 24 03	0.0145	321	22137.93
B AC 20 24 04	0.036	697	19361.11
B AC 20 24 05	0.0253	480	18972.33
B AC 20 24 06	0.0192	490	25520.83
B AC 20 24 07	0.0192	415	21614.58
B AC 20 24 08	0.0192	444	23125
B AC 20 24 09	0.0267	559	20936.33
B AC 20 24 10	0.0192	360	18750
B AC 20 24 11	0.0145	229	15793.1
B AC 20 24 12	0.0365	778	21315.07
B AC 20 24 13	0.024	368	15333.33
B AC 20 24 14	0.0587	877	14940.37
B AC 20 24 15	0.069	965	13985.51
AVE			19805.17
STD			3529.253

Table B.3.29: B-AC-50mg-24HR

B - AC- 50mg - 24HR			
SAMPLE		NUMBER	CONC.
B AC 50 24 01	0.0365	558	15287.67
B AC 50 24 02	0.024	447	18625
B AC 50 24 03	0.0282	578	20496.45
B AC 50 24 04	0.0267	556	20823.97
B AC 50 24 05	0.0267	596	22322.1
B AC 50 24 06	0.0192	412	21458.33
B AC 50 24 07	0.0145	284	19586.21
B AC 50 24 08	0.0282	658	23333.33
B AC 50 24 09	0.0267	456	17078.65
B AC 50 24 10	0.0267	499	18689.14
B AC 50 24 11	0.0192	559	29114.58
B AC 50 24 12	0.0145	225	15517.24
B AC 50 24 13	0.0365	698	19123.29
B AC 50 24 14	0.024	335	13958.33
B AC 50 24 15	0.02	257	12850
AVE			19217.62
STD			4109.725

Table B.3.30: Control – B – 24HR

Control - B - 24HR			
SAMPLE		NUMBER	CONC.
Ctrl B 24HR 01	0.0282	667	23652.48
Ctrl B 24HR 02	0.0267	848	31760.3
Ctrl B 24HR 03	0.0145	874	60275.86
Ctrl B 24HR 04	0.036	748	20777.78
Ctrl B 24HR 05	0.0253	945	37351.78
Ctrl B 24HR 06	0.0192	687	35781.25
Ctrl B 24HR 07	0.0192	501	26093.75
Ctrl B 24HR 08	0.0192	297	15468.75
Ctrl B 24HR 09	0.0192	645	33593.75
Ctrl B 24HR 10	0.0192	447	23281.25
Ctrl B 24HR 11	0.038	412	10842.11
Ctrl B 24HR 12	0.0192	941	49010.42
Ctrl B 24HR 13	0.0192	398	20729.17
Ctrl B 24HR 14	0.0171	365	21345.03
Ctrl B 24HR 15	0.0251	657	26175.3
AVE			29075.93
STD			12839.97

B.4 Images form SEM

Below are some images from the SEM before processing are counting.

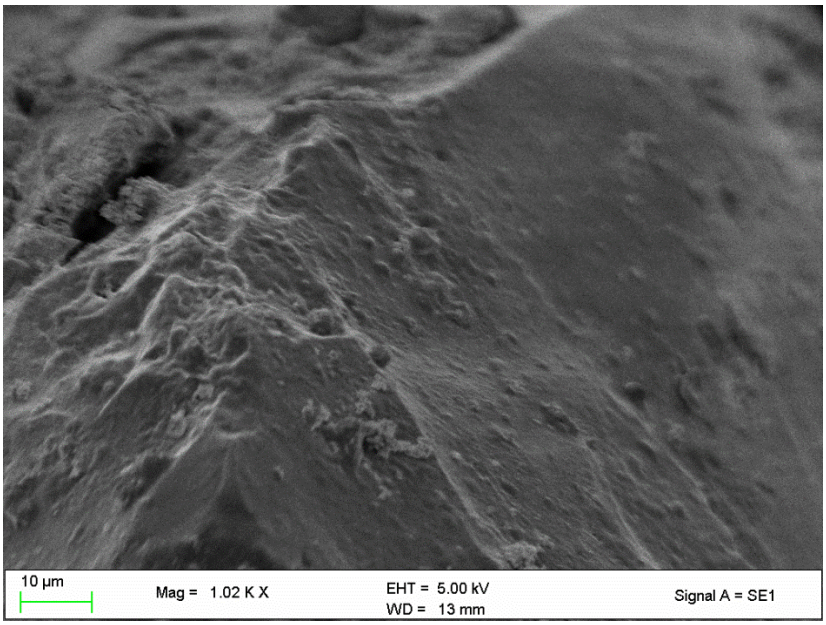

Figure B.1- SEM image

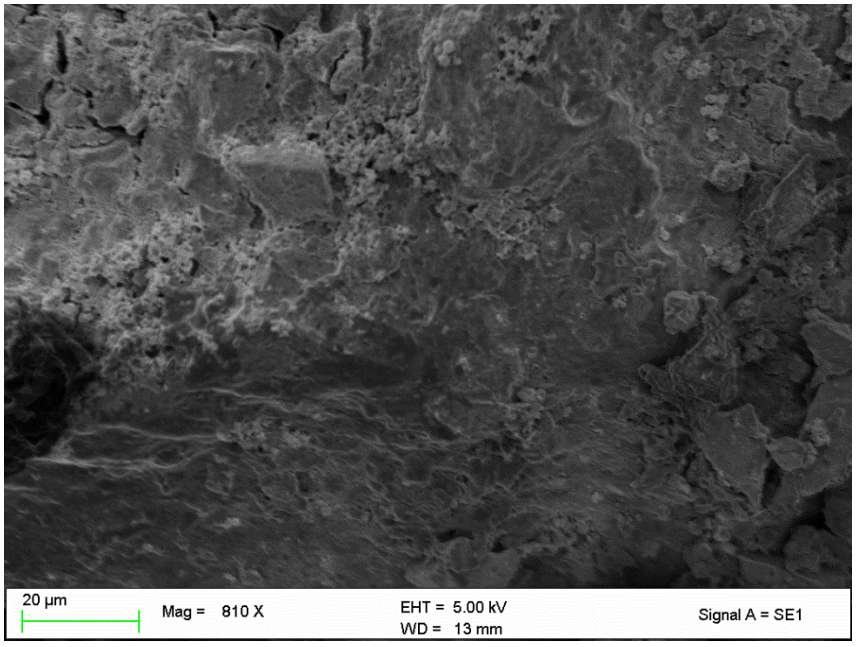

Figure B.2- SEM image

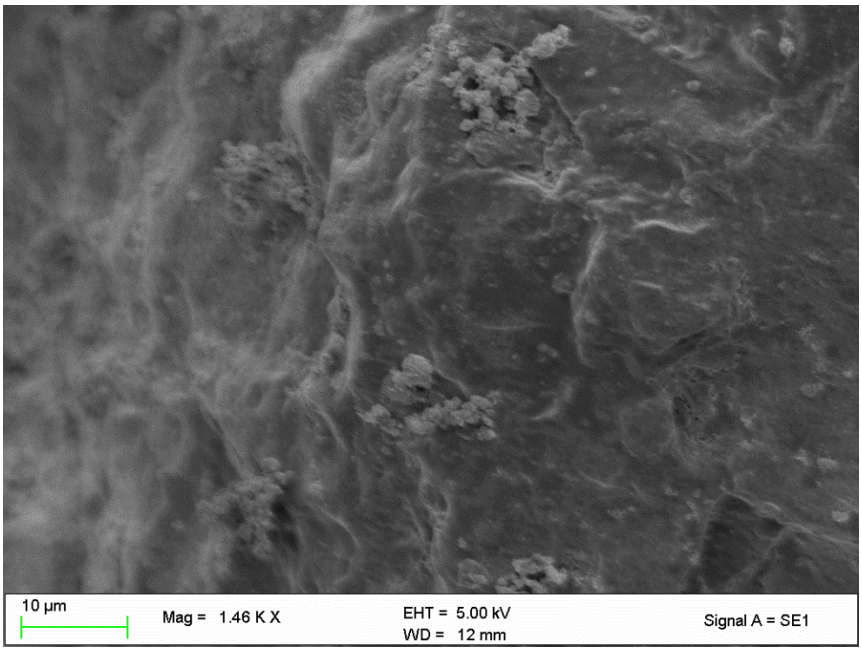

Figure B.3- SEM image

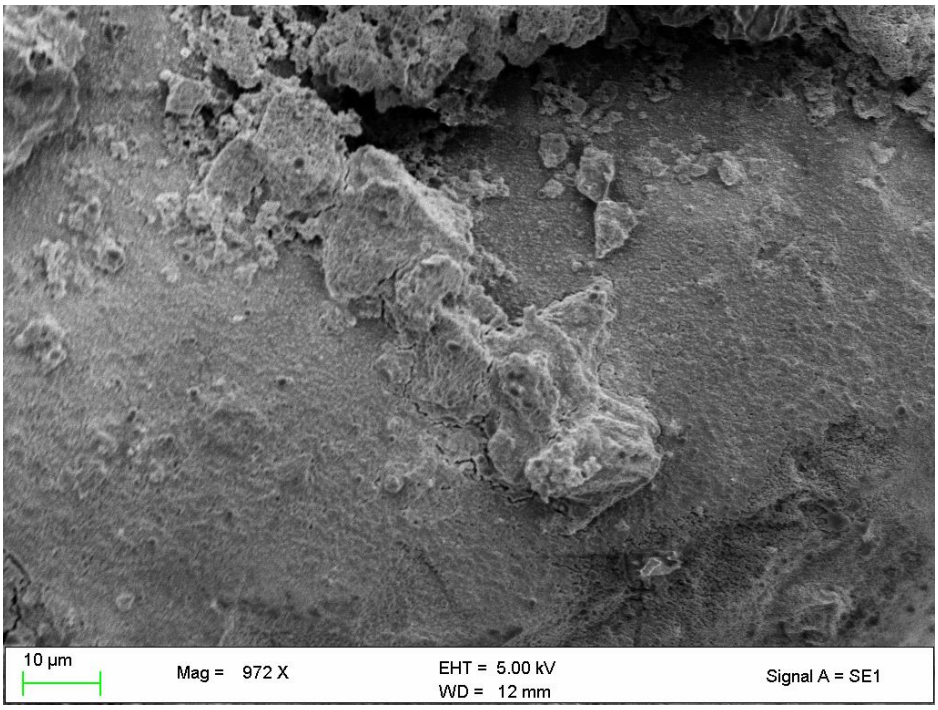

Figure B.4- SEM image

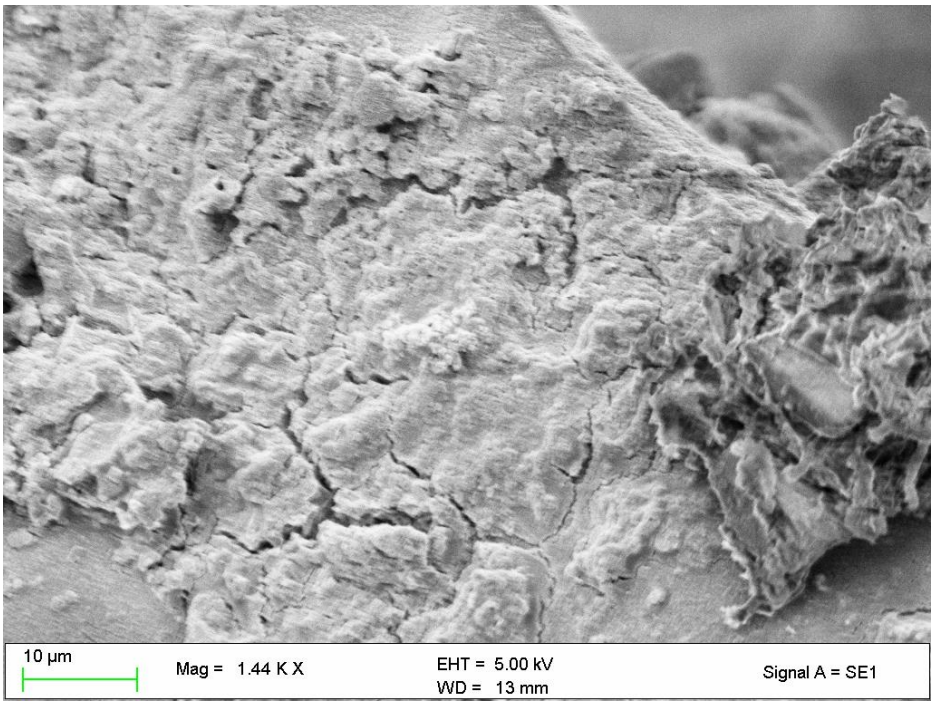

Figure B.5- SEM image

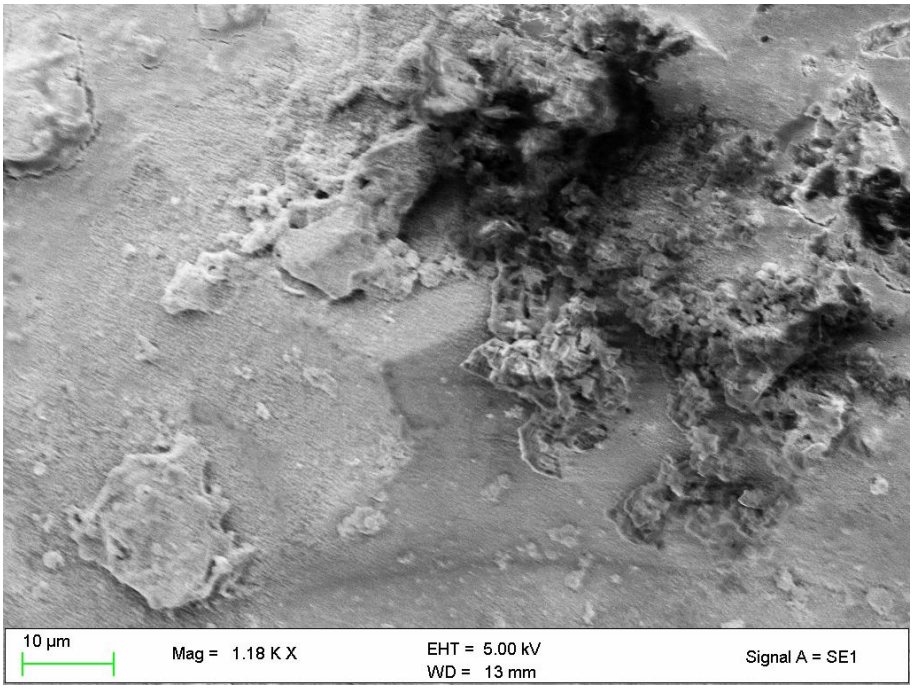

Figure B.6- SEM image

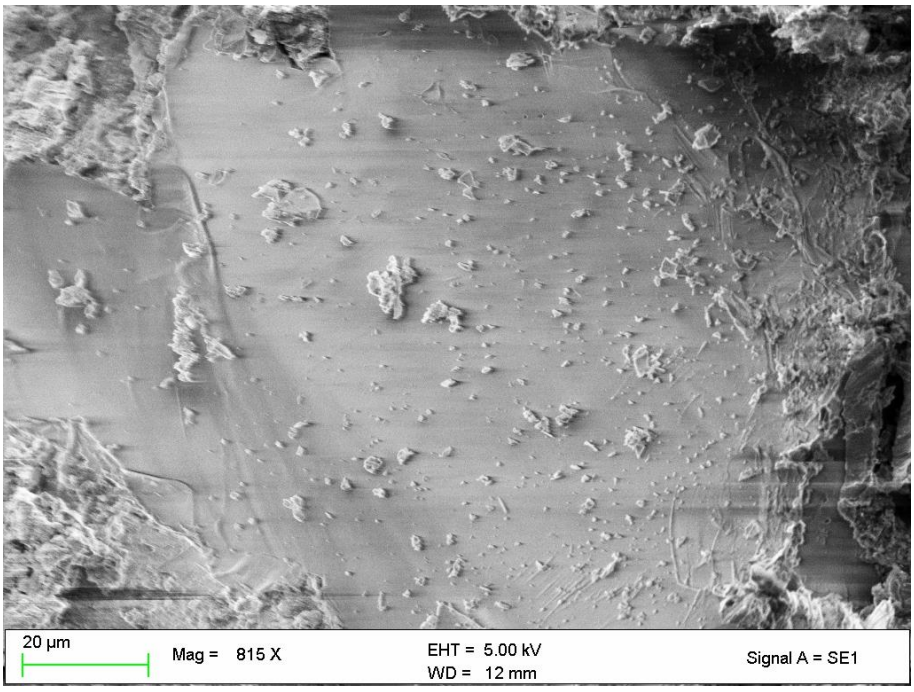

Figure B.7- SEM image

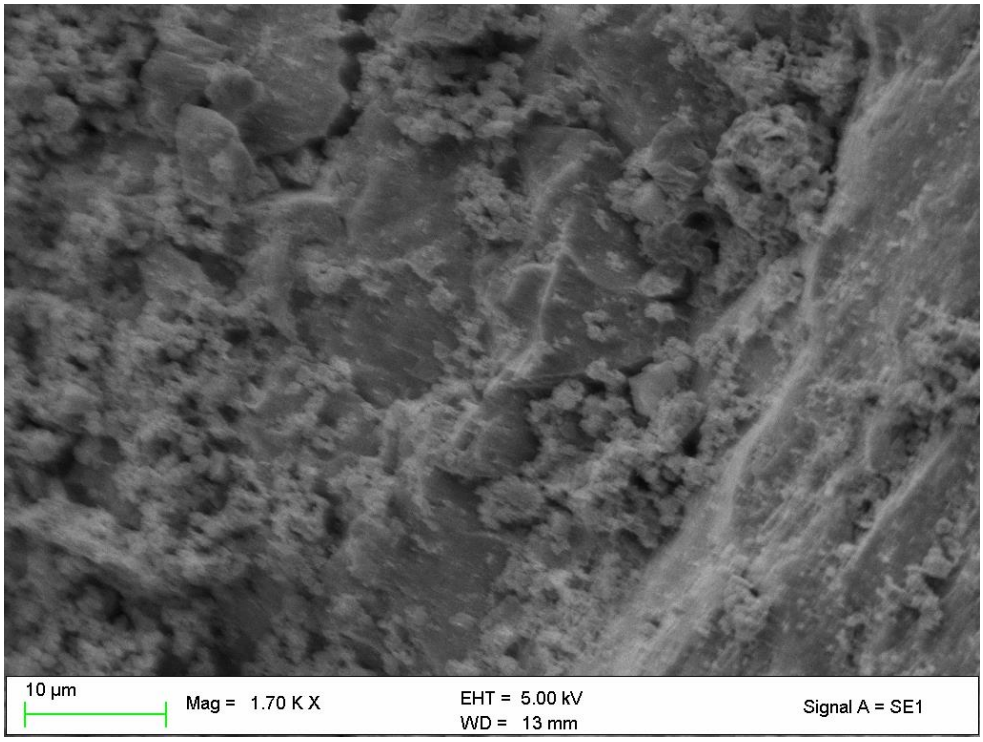

Figure B.8- SEM image

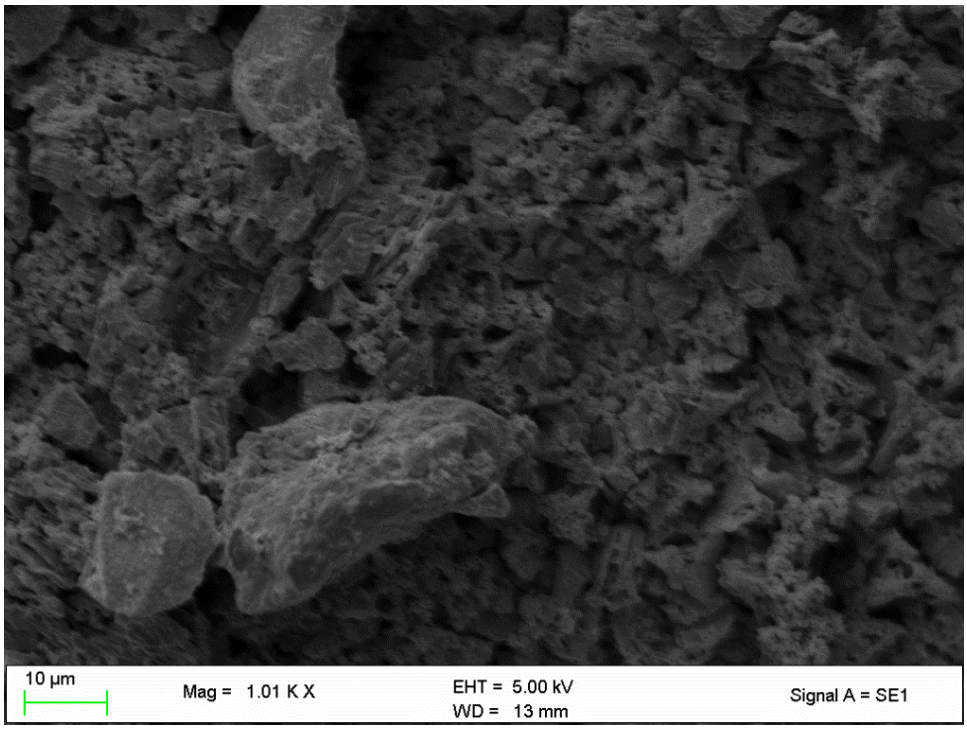

Figure B.9- SEM image

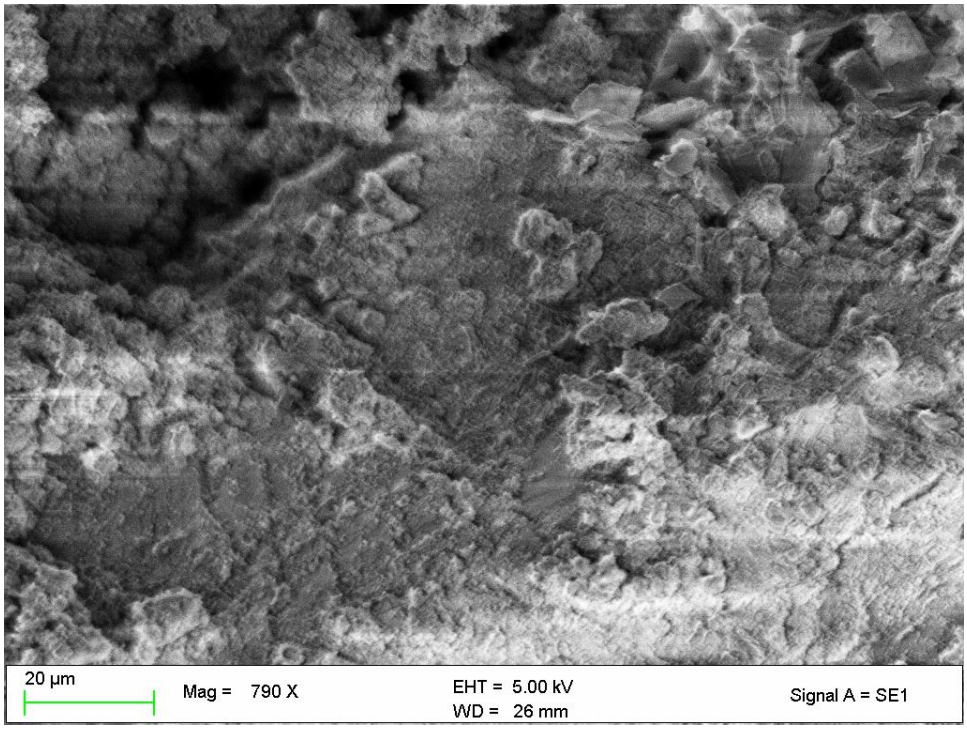

Figure B.10- SEM image

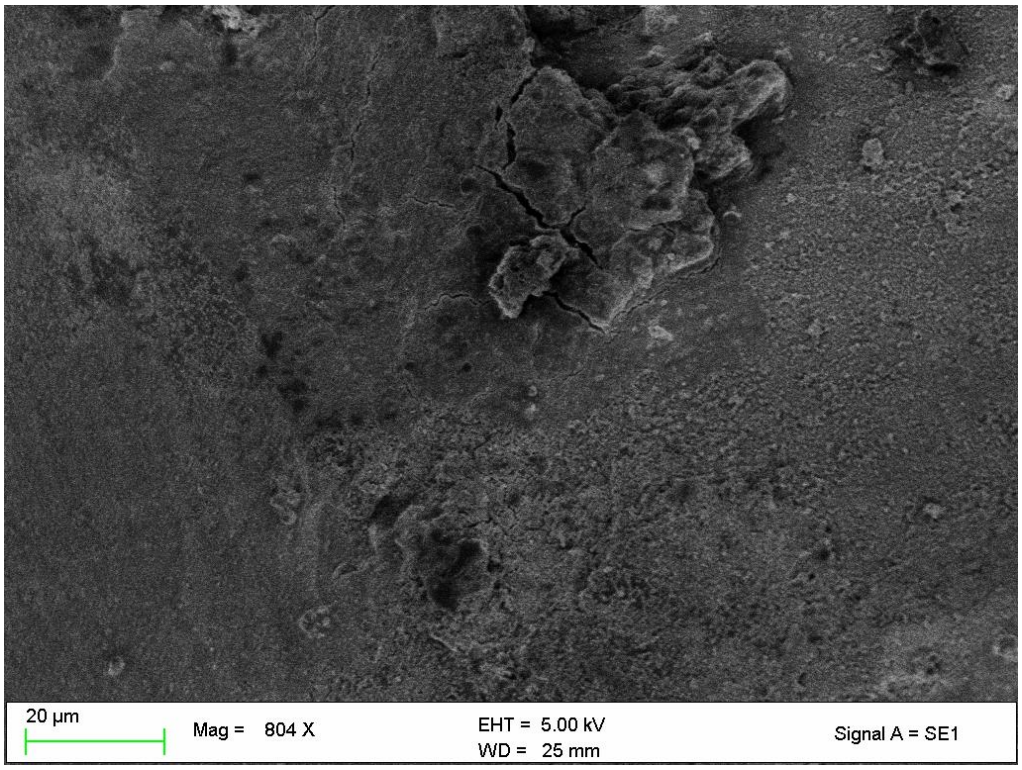

Figure B.11- SEM image

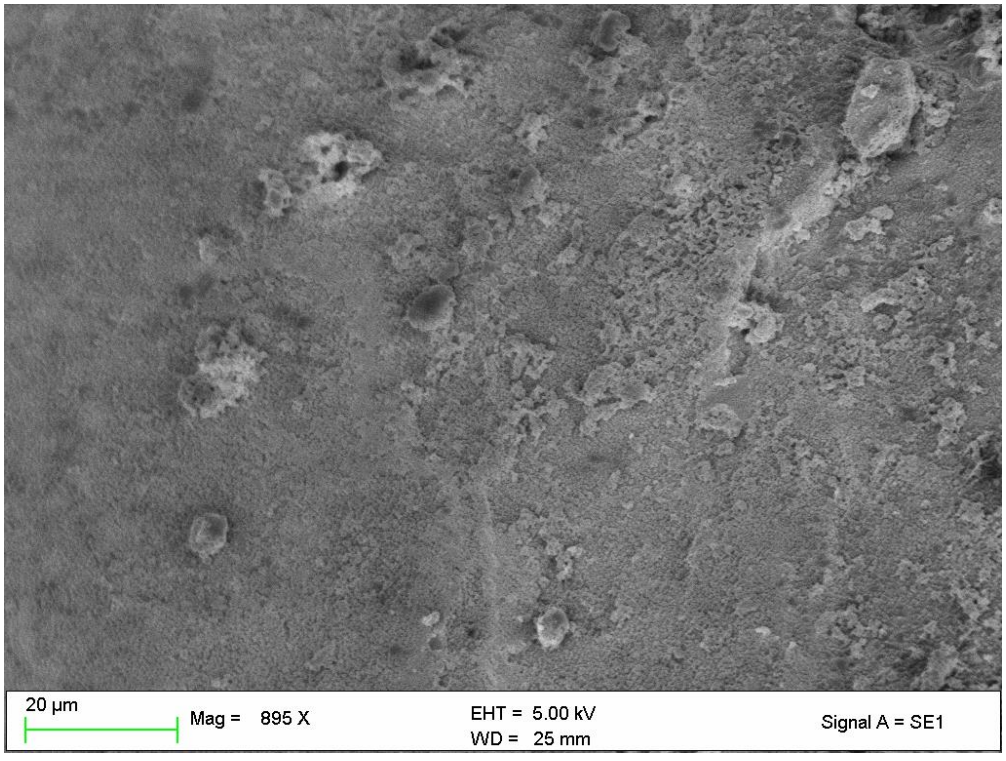

Figure B.12- SEM image

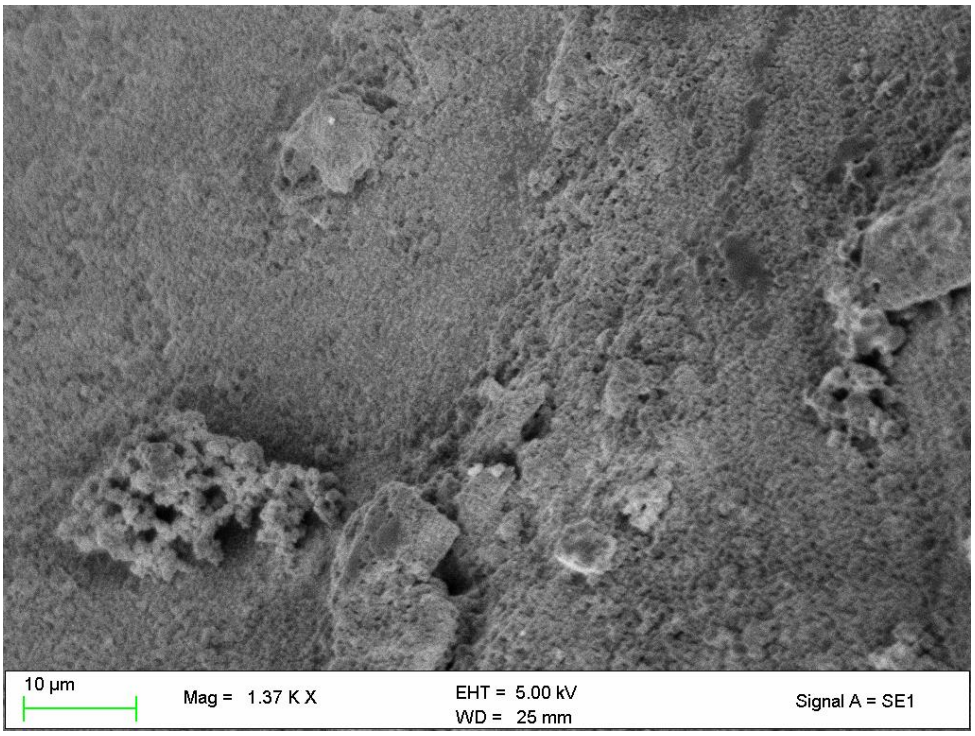

Figure B.13- SEM image

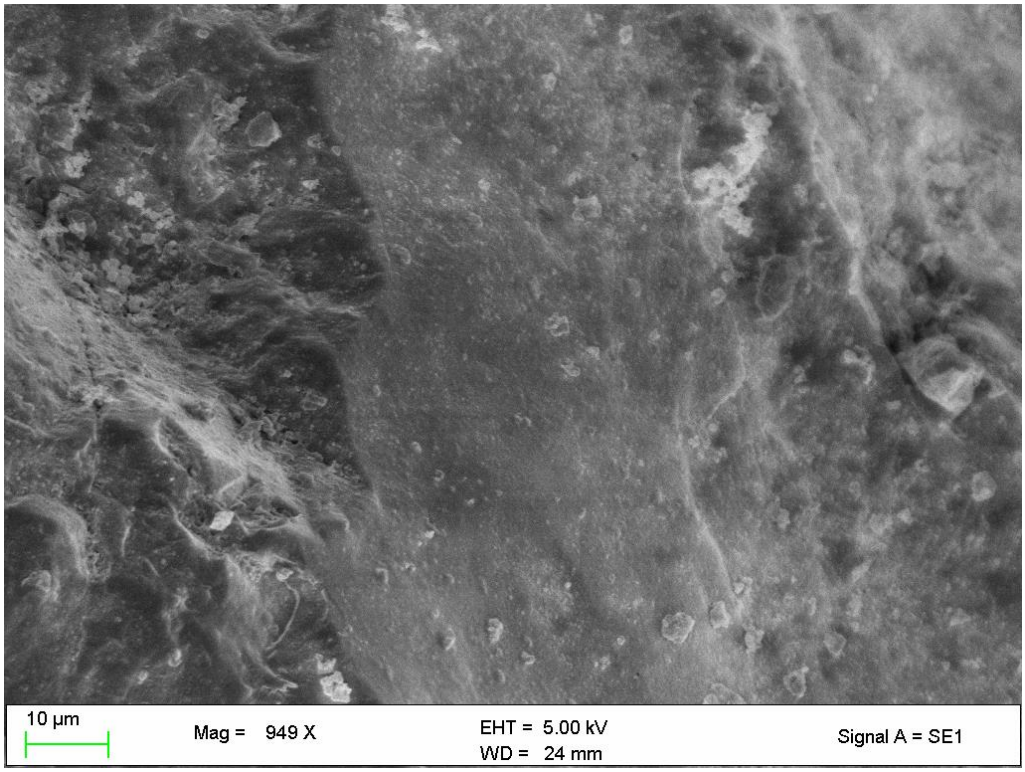

Figure B.14- SEM image

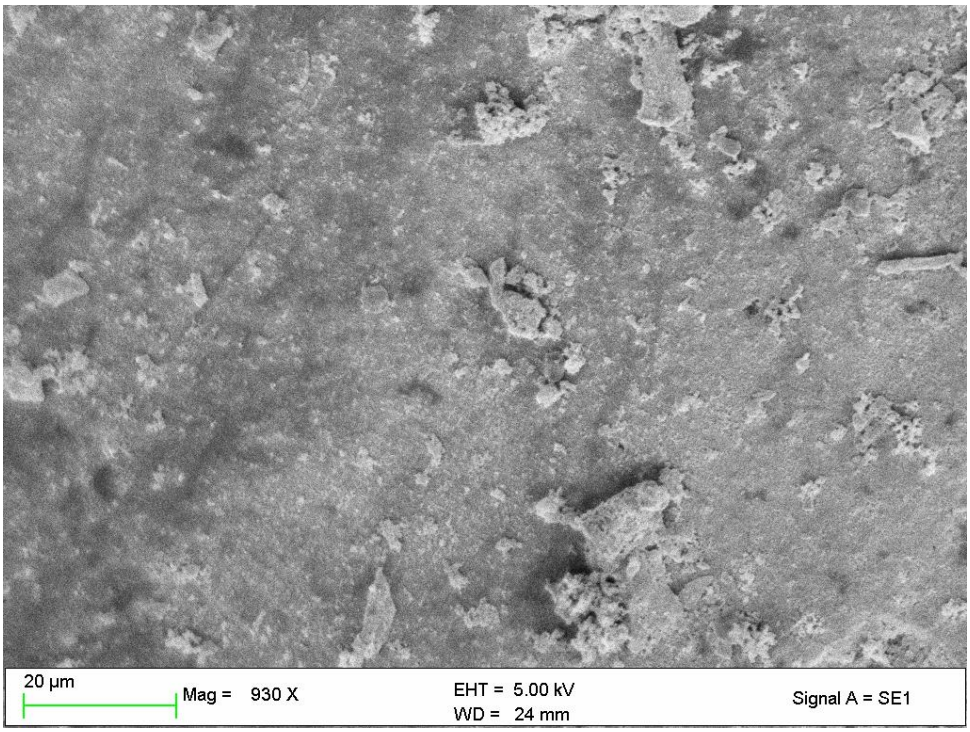

Figure B.15 - SEM image

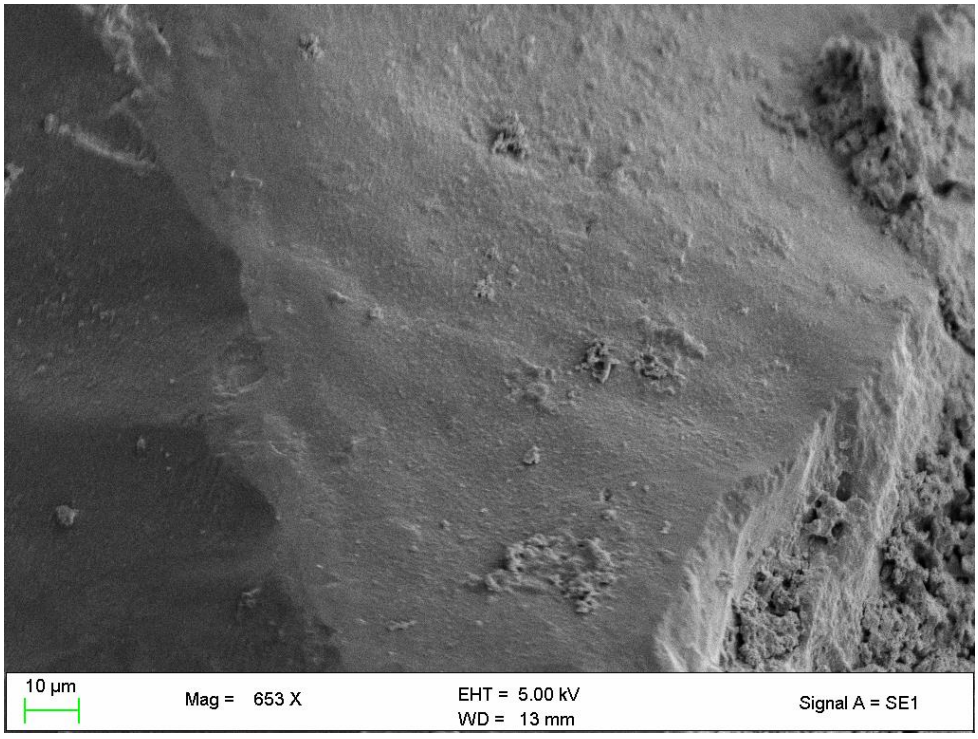

Figure B.16 – SEM image