

THE PENNSYLVANIA STATE UNIVERSITY  
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOLOGY

A CULTURE-DEPENDENT APPROACH TO EXAMINE THE EFFECTS OF SILVER IONS  
ON BACTERIAL COMPOSITIONS WITHIN LOCAL STREAMS

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SPRING 2020

A thesis  
submitted in partial fulfillment  
of the requirements  
for a baccalaureate degree  
in Biology  
with honors in Biology

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## ABSTRACT

Our society has become more aware of the abundance of bacteria which is evident by the increase in antimicrobial products over the past two decades. A commonly used agent in many antimicrobial products is silver ions due to its multifactorial approach to killing a wide range of microorganisms. Most of the research concerning silver ions has focused on its antimicrobial effectiveness and considerably less research has been done on any effect of the over-usage of silver in the environment. Thus, the goal of our study is to determine whether silver is affecting bacterial ecosystems within our local waterways. For the study, collections from surrounding streams were exposed to coupons either coated with silver zeolite or a non-silver coating. After 48 hours of exposure, the samples were transferred to a filter membrane through vacuum filtration and plated onto: tryptic soy agar (TSA), modified mTec agar (Mtec), an m-enterococcus agar (ME), and Carbapenem-resistant Enterobacteriaceae agar (CRE). During 0, 24, and 48 hours of incubation, the concentration of silver was measured using an ICP-MS. The silver treatment showed an average silver concentration of 40 +/- 20 ppb was released after 48 hours. The culture-dependent bacterial results showed a decrease in growth with all samples treated with silver ions.

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## ACKNOWLEDGEMENTS

I would like to thank Dr. Beth Potter, my thesis supervisor. Without her none of my research would had been possible. She has provided me all the best experiences/ lessons an undergraduate research student could ask for. We have worked on research together for three years and during that time I have learned so much more than I would have by sitting in a classroom. The lessons she has taught me, will stay with me throughout the rest of my career. She has prepared me more for the future more than any other person. Because of her suggestions and guidance towards writing this manuscript, I have never been more ready for my future. Additionally, I would like to thank Dr. Michael Campbell, my honors advisor, for his suggestions and guidance towards this manuscript. I would also like to thank Dr. Deborah Aruquete for her guidance in the field for silver testing, Dr. Michael Rutter for his insights into the statistical analysis, Jerry Magraw for his lessons and experience with the ICP-MS, Dr. Jason Bennett for his suggestions towards the silver results, and Amber Stillwell (currently at the Tom Ridge Environmental Center) for teaching me the membrane filtration technique. This research was made possible, in part, through funding provided by the Penn State Erie – The Behrend College Undergraduate Student Research Fellowships and Schreyer Honors Grant. Furthermore, I would like to thank the Schreyer Honors College for this opportunity to express my ambitions towards research. Lastly, I would like to thank my friends and family for the support they have provided me throughout my research.

## Chapter 1

### INTRODUCTION

A metal that has been used since the beginning of recorded history, silver, is used for many of its properties including conductivity, malleability, value, shine, and antimicrobial activity (Russell, 1994). Silver's antimicrobial activity has been used for centuries and changed the lives of the Greek and Roman empires when it was first used to preserve liquids and foods by lining the insides of the containers (Franke, 2007). In the middle ages, silver nitrate was first medically used to treat chronic wounds and ulcers (Franke, 2007). It was in the 19th century that the antimicrobial activity of silver ions was recognized (Alexander, 2009; Franke, 2007; Russell and Hugo, 1994). One of silver's best-known medical applications was the treatment of burn wounds using silver salts, primarily silver nitrate (Alexander, 2009; Franke, 2007; Russell and Hugo, 1994). Silver's medical significance prospered into the 20th century with William Stewart Halsted being one of the first American surgeons to advocate the use of silver foil for wound dressing and silver sutures during surgical incisions to prevent infections (Alexander, 2009). Shortly after, in 1920s, colloidal silver was accepted by the US Food and Drug Administration (FDA) to be an effective method in wound management (Alexander, 2009; Chopra, 2007). Between the 1900 and 1940s, silver was also used intravenously and in its colloidal form to treat tens of thousands of patients with various infectious diseases (Alexander, 2009). However, the discovery of antibiotics in the 1940s, such as penicillin, caused silver's antibacterial properties to be largely overlooked. (Chopra, 2007; Alexander, 2009). Now with the emergence and accumulation of antibiotic-resistant bacteria, many silver-based products have been introduced to prevent/treat infections (Silver et al., 2006; Chernousova & Epple, 2012). Silver sulfadiazine was

developed in the 1980s to be used in wound treatment cream and it largely replaced silver salts including silver nitrate, lactate, picrate, and sulphate (Burrell, 2003; Franke, 2007; Russell and Hugo, 1994). Beyond medical treatments, silver has now been incorporated into many commercial and personal products such as water filters, deodorant, cosmetics, and children's sippy cups (Silver et al., 2006; Chernousova & Epple, 2012).

The widespread use of silver began when it was realized that silver ions could be released from silver salts and metallic silver after their incorporation into thermoplastic polymers (Egger et al., 2009). This was quickly extended with the release of silver ions from other inert matrices including glass, ceramic, zeolite, and nanoparticles (Egger et al., 2009; Kawahara, 2000; Matsumura et al., 2003). Zeolite is a porous crystalline material composed of hydrated sodium aluminosilicate that releases  $\text{Ag}^+$ , but because of its high affinity for silver the zeolite releases the ions at lower concentrations and over a sustained period (Kawahara, 2000; Matsumura et al., 2003). Zeolite exhibits low toxicity to humans and can be incorporated into powders capable of coating surfaces used for toys, kitchen wares, and medical supplies (Hotta et al., 1998; Matsumura et al., 2003). As of today, the newest and most leading form of silver is nanosilver/silver nanoparticles (Choi et al., 2018). Like zeolite technology, silver nanoparticles have low toxicity towards humans and release silver ions at low concentrations over time (Choi et al., 2018). The antibacterial effect of silver nanoparticles has been observed to be both size and shape-dependent (Morones et al., 2005; Pal et al., 2007). Silver nanoparticles with at least one dimension less than 100nm exhibit unique physicochemical properties such as higher catalytic and binding strength due to the increase in surface area (Choi et al., 2008; Morones et al., 2005; Pal et al., 2007). Different nanoparticle shapes also contribute to surface area, in that, they manifest more or less interactable surfaces/facets (Morones et al., 2005). Both of these



attributes give rise to more effective antibacterial properties by their increasing membrane interaction and their ability to enter the cell (Choi et al., 2008; Morones et al., 2005; Pal et al., 2007). Although the various types of silver compounds/materials can have their own intrinsic antimicrobial effects, the most prominent property of each is the release of silver ions, which have the largest antibacterial impact (Chernousova & Epple, 2012, Luoma, 2008).

Silver ions present many advantages in comparison to antibiotics. First, silver's antimicrobial influence has been described as oligodynamic, meaning it requires very small concentrations to be effective (Russel and Hugo, 1994). Studies have shown silver ions to be effective against bacteria as low as  $1\mu\text{M Ag}^+$  (108 parts per billion) (Chopra, 2007; Li, et al., 2017). The risks of silver to human health are low and only non-lethal effects arise after prolonged exposure, therefore, the oligodynamic properties of silver further reduce the health risks (Mijnendonckx et al., 2013). Second, it has been shown to be effective against a wide range of microorganisms including viruses, yeast, fungi, as well as both Gram-positive and Gram-negative bacteria (Marambio-Jones & Hoek, 2010).

Finally, and of most significance, silver does not have just one target to kill/inhibit the reproduction of bacteria like antibiotics; rather it has a multifactorial approach (Figure 1 from Mijnendonckx et al. 2013). In terms of structural effects, silver has been observed to create "holes" in the bacterial surface by damaging the cell's plasma membranes (Feng et al., 2000; Sondi & Salopek-Sondi, 2004; Stoimenov et al., 2002). This leads to the cells inability to regulate transport and significantly increases permeability which eventually leads to the leakage of intracellular components (Feng et al., 2000; Sondi & Salopek-Sondi, 2004; Stoimenov et al., 2002). The components released are that of reducing sugars and proteins, important in maintaining the cell's overall metabolism and normal functions. Leakage can be significant

causing the cytoplasm to shrink to such a degree that the cytoplasmic membrane detaches from the cell wall (Feng, et al., 2000; Jung et al., 2008; Soni & Salopek-Soni, 2004; Stoimenov et al., 2002).

Coordinating further destruction within the cytoplasmic membrane, silver can also target several membrane-bound proteins. The lethal interaction is mediated by thiol groups and these are found in proteins involved in respiration as well as protein, metabolite and electron transport (Feng, et al., 2000; Marambio-Jones and Hoek, 2010; Mijndonckx et al., 2013; Schreurs and Rosenberg, 1982). Carrier proteins, responsible for regular exchange of metabolites between membranes, are altered to facilitate the efflux of important metabolites such as potassium, phosphate, mannitol, succinate, glutamine, and proline out of the cell (Jung et al., 2008; Lok et al., 2006; Schreurs and Rosenberg, 1982). The inhibition of respiration and electron transport, compounded with the significant loss in intracellular potassium, leads to a collapse of the proton motive force, a vital component to the cells overall energy status, resulting in a de-energizing of the cell membrane, decrease in ATP levels, and ultimately cell death (Lok et al., 2006; Mijndonckx et al., 2013).

Silver is not relegated to affecting just membrane proteins; silver ions can enter the bacterial cytoplasm and interact with numerous cytoplasmic proteins via their thiol groups (Mijndonckx et al., 2013; Stoimenov et al., 2002). A significant consequence of these cytoplasmic interactions is the generation of reactive oxygen species (ROS), specifically superoxide radical ( $O_2^-$ ) (Mijndonckx et al., 2013; Park et al., 2009). The generation of these ROS has been proposed to be due to silver's thiol interaction with respiratory chain enzymes or superoxide scavenging enzymes (Marambio-Jones and Hoek., 2010). ROS are short lived and toxic to cells because of the oxidative stress they induce by damaging proteins, DNA, RNA, and

lipids (Choi et al., 2018). In a normal cell, ROS are produced in small concentrations by respiration, but these levels are maintained by antioxidant defenses such as the glutathione/glutathione disulfide (GSH/GSSG) ratio which regulates normal ROS levels in the cell (Marambio-Jones and Hoek., 2010). Silver has been shown to inactivate these defenses by binding to GSH directly or with other GSH maintenance enzymes (Marambio-Jones and Hoek., 2010).

Silver has one last target within the cytoplasm, the bacterial genome comprised of deoxyribonucleic acid (DNA). A macromolecule that is vital to all cells, DNA, stores the genetic information responsible for its own replication, as well as, the cell's division and normal functions. While in a bacterial cell, silver ions complex with nucleic acids, preferentially interacting with nucleosides, rather than the phosphate groups, where it causes DNA condensation and increases DNA mutation, which in turn, interferes with DNA replication (Jung, et al. 2008; Marambio-Jones and Hoek., 2010). Unlike eukaryotic cells, DNA within a bacterium is not protected by a nucleus, and, because of its shorter lifespan of around 20 minutes, bacteria would experience more of an effect, from silver, than eukaryotic cells (Vazquez-Muñoz et al., 2017).

Thus, the rise of antimicrobial silver products given silver's superior antimicrobial effectiveness is not surprising. Silver nanoparticles (AgNPs) have now become the most used nanomaterial in medical (dressings and catheters), consumer (soaps, socks), and industrial (food packaging, building materials) products (Blaser et al., 2008). A point of concern during the production of AgNPs is that silver ions can enter the environment via several different pathways (Figure 2 adapted from Blaser et al., 2008) (Blaser et al., 2008, Kim et al., 2010). It has been estimated that 15% of silver in the European Union's water supply in 2015 was introduced from

antimicrobial products (Blaser et al., 2008). Silver's progression into the environment begins at its production where silver ore is mined and refined (Lanzano et al., 2006). Next, manufacturing/fabrication involves the incorporation of silver into products, followed by the intended use of the product, and eventually to the recycling/disposal of the product (Lanzano et al., 2006). At each of these stages, silver is released into wastewater, and, when left untreated, it enters the aquatic ecosystem directly (Blaser et al., 2008, Lanzano et al., 2006). Alternatively, wastewater treatment plants (WWTPs) take in wastewater and silver is combined with sewage sludge by reacting with reduced sulfur (Kim et al., 2010). However, studies have shown that approximately 10% of the silver nanoparticles will not combine with the sludge and enter surface waters through the WWTP effluent (Blaser et al., 2008; Colman et al., 2014, Kim et al., 2010). The remaining silver in the sludge is either disposed of in solid waste landfills, applied to agricultural soils as fertilizer, or incinerated at a thermal waste treatment plant (Blaser et al., 2008). With both fertilizer and landfills, silver has the likelihood of leaching into the soil and groundwater (Blaser et al., 2008). In 2009, the United States Environmental Protection Agency (EPA) sampled 74 WWTPs to statistically estimate sewage sludge silver concentrations of WWTPs that are indicative of silver levels found in sewage throughout the United States and found the analyzed sludge to contain a total concentration range of 1.9 to 856 mg kg<sup>-1</sup> (1900 to 856000 ppb) (Kim et al., 2010). The results of this leaching and overall silver accumulation can have a significant effect on aquatic ecosystems, effecting eukaryotic and prokaryotic organisms (Chernousova & Epple, 2012, Luoma, 2008).

Silver has been said to be one of the most toxic metals regarding its acute toxicity to freshwater organisms, and several studies have focused on fish and invertebrates (Janes and Playle, 1995; Luoma, 2008; Wood, 2011; Wood et al., 1996; Zhou et al., 2005). Its interaction

with freshwater fish has been primarily studied with trout and has shown symptoms resembling that seen with low pH exposure, i.e. decrease in  $\text{Na}^+$  and  $\text{Cl}^-$  levels within blood, fluid volume disturbances, and circulatory failure (Wood, 2011; Wood et al., 1996). However, the mechanism behind the symptoms differ in that silver inhibits regulation of  $\text{Na}^+$  and  $\text{Cl}^-$  by targeting  $\text{Na}^+/\text{K}^+$ -ATPase and carbonic anhydrase activity in ion-transporting cells (ionocytes), predominately in the gills (Wood, 2011). It has been observed that silver works on  $\text{Na}^+/\text{K}^+$ -ATPase by noncompetitively inhibiting  $\text{Na}^+$ , but instead competing with  $\text{Mg}^{2+}$  that normally binds to the  $\text{Mg}^{2+}$  activation site located on the intracellular domain of the  $\alpha$ -subunit (Wood, 2011). In addition, silver also inhibits carbonic anhydrase through an unknown mechanism (Wood, 2011). Carbonic anhydrase is an enzyme responsible for the formation of two counterions,  $\text{H}^+$  and  $\text{HCO}_3^-$ , which assist in the uptake of  $\text{Na}^+$  and  $\text{Cl}^-$  (Wood, 2011).

Other studies have directed their attention to invertebrate species lower on the food web, such as daphnids and algae (Chernousova & Epple, 2012; Griffitt et al, 2008; Luoma, 2008; Ratte, 1999; Wood, 2011). Daphnids, specifically *Daphnia magna* and *Daphnia pulex*, have been observed to be approximately 10-fold more sensitive than fish to silver ions due to their smaller size and filtering-feeding (Griffitt et al, 2008; Luoma, 2008; Ratte, 1999; Wood, 2011). Algal species were also shown to have a reduction in growth rate (Ratte, 1999). Not only were algae species (*Pseudokirchneriella subcapitata*, *Selenastrum capricornutum*) observed to be susceptible to silver, but *S. capricornutum* absorbed silver into the cell walls (Griffitt et al, 2008; Ratte, 1999) The effect on these lower level organisms can have high ecological impacts for the rest of the food web (Griffitt et al, 2008; Ratte, 1999).

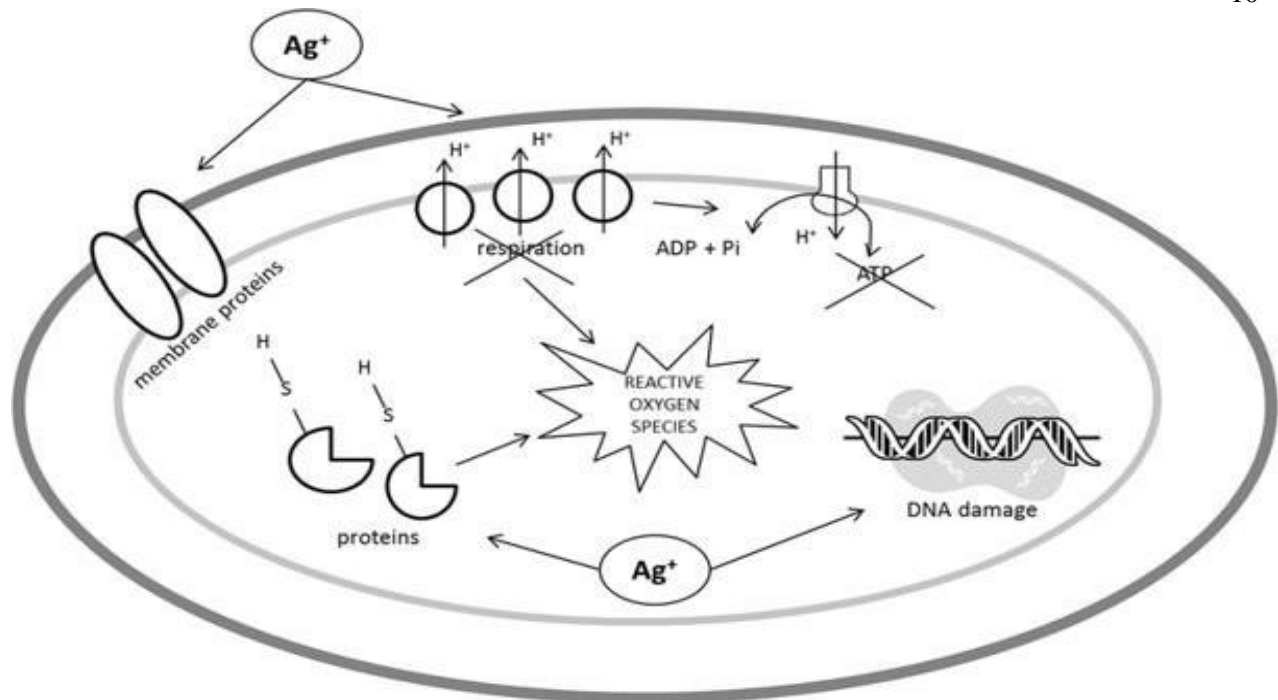
Regarding prokaryotic microorganisms, little research has focused on environmental bacterial populations. Fabrega et al. commented in their review article: “Exposure of natural

bacterial communities of microorganisms to silver nanoparticles is likely in surface waters and wastewater treatment plants, yet little research has been done on silver or silver nanoparticles and natural bacterial communities” (2011). Two studies were found and showed that nitrifying bacterial populations in wastewater treatment facilities are susceptible to silver nanoparticles and that the susceptibility is higher than that determined for cultures of *E. coli* (Choi and Hu, 2008; Choi et al., 2008). It has been demonstrated that silver has an inhibitory effect on enzymes of nitrifying bacteria during the Phosphorus-, Sulfur-, and Nitrogen-cycles (Domsch KH, 1984). Such powerful effects on nutrient cycling could have significant effects on the efficiency of wastewater treatment facilities and natural soil ecosystems (Domsch KH, 1984; Fabrega et al., 2011)

Ultimately, there is concern that overuse of silver products and the confirmation of silver leaching into the environment will lead to resistance issues. While it was originally thought that silver’s multifactorial effects on organisms would make resistance unlikely, silver resistance has been located on plasmids within bacterial species isolated in hospitals, silver mines, and silver-contaminated areas (Franke, 2007). The presence of silver resistance genes on mobile elements, such as plasmids, allows for horizontal gene transfer, increasing the risk of widespread resistance (Mijnendonckx et al., 2013). The limited literature surrounding the effects of silver ions on bacterial populations within natural aquatic ecosystems has inspired our research to fill this gap of knowledge. The location of the Penn State Behrend campus is advantageous in studying our local environment and streams of the Wintergreen Gorge. The experiment was to examine how the bacterial compositions within three local streams was altered with exposure to silver-zeolite as compared to a control, non-silver treatment. In order to detect the concentration of silver ions released by the silver-zeolite, silver concentrations from both the streams and silver- and control-

treated coupons were determined by Induced Coupled Plasma Mass Spectroscopy (ICP-MS).

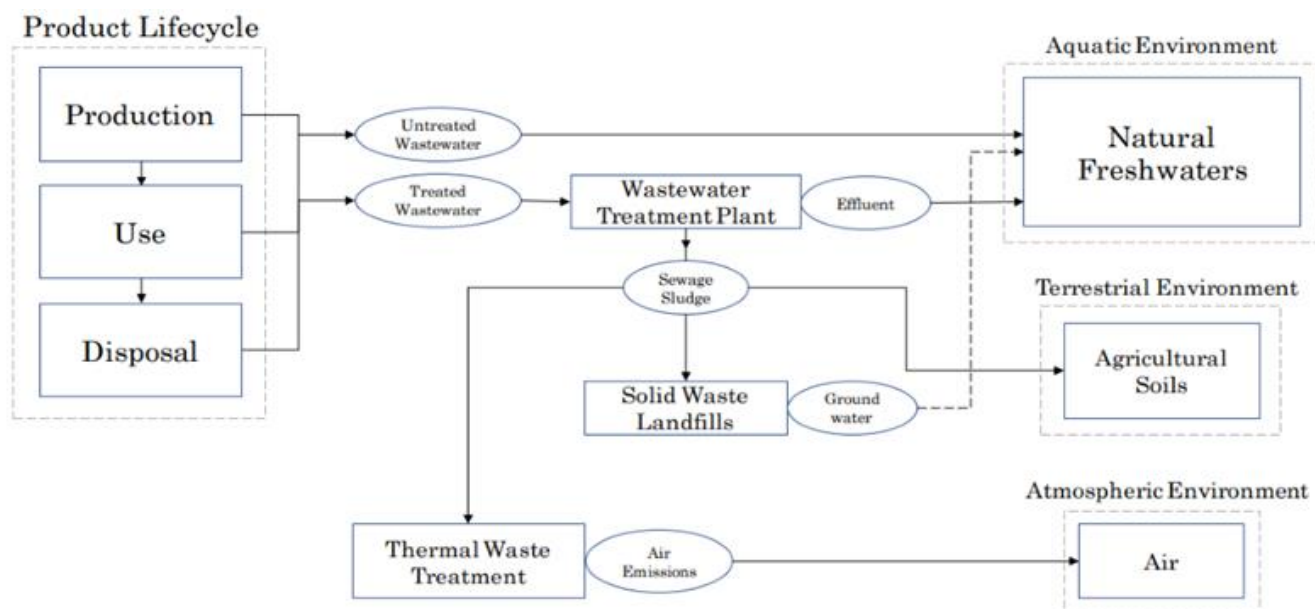
Given a review of the literature and some preliminary evidence, we hypothesized that bacterial composition would be affected due to susceptibility to silver ions.



**Figure 1: The Antibiotic Effects of Silver Ions**

Silver interacts with membrane proteins, inhibiting respiration and electron transfer. While inside the cell, silver ions interact with DNA, proteins, and induce reactive oxygen species production (Mijnendonckx et al. 2013).





**Figure 2: Silver's Ability to Enter the Environment**

This simplified diagram depicts how each stage of a silver product's lifecycle leads to the accumulation of silver in the environment (Blaser et al., 2008).

## **Chapter 2**

### **MATERIALS AND METHODS**

#### **Collection Sites of Stream Water**

Three different streams located within the Wintergreen Gorge and/or run through Penn State Behrend were used for water collection sites. The first site, reference, is located deep upstream within the Wintergreen Gorge at (42.108715, -79.984119) and would be expected to receive highway runoff from Interstate-90. TroutRun is beneath the athletic bridge leading up to Behrend fields (42.120191, -79.984927) and receives runoff from the athletic fields and potentially the Bayfront Connector. Glen Hill is next to Turnbull Hall, the psychology building on the Penn State Behrend Campus (42.121948, -79.985848) and experiences runoff from the campus. For each stream, water samples were collected in triplicate. Additionally, ecological parameters were taken, and silver analysis was done as well as at various stages of the experiment, including the time of collection and during sample incubation. A total of four trials were done and started on 8/12/2019, 10/7/2019, 10/21/2019, and 11/4/2019 respectively and between the times of 7:00am - 10:00am.

#### **Stream Sample Collection/ Processing**

Water samples were collected in Whirl-Pak bags with approximately 330 mls in each. The bags were handled with sterile gloves, labelled, sealed, and immediately placed into a mini cooler upon collection. In the lab, 160 mls was separately transferred to two deep Petri-dishes (140mm x 20 mm), one containing a silver (Agion zeolite) coated coupon and another containing

a control-coated coupon, both of which were previously sterilized with 70% ethanol (Advanced Finishing U.S.A). Immediately following transfer, a 5 ml sample was removed from both the silver- and control Petri dishes for silver analysis. The Petri dishes were covered in aluminum foil to prevent light exposure and transferred into an orbital shaker incubator set to 50 rpm and 25 °C for 48 hours.

After the water samples were incubated for 48 hours, the water was transferred into sterile conical tubes. In order to create statistically relevant bacterial counts, samples were serially diluted. Dilutions were based on test samples done before official trials were run. Throughout the experiment, modifications were made to the dilutions to maintain plating efficiency. In general, the tryptic soy agar (TSA), an all-purpose agar, had a larger range of dilutions for both treatments compared to that of the more selective agars used. Once diluted, all the conical tubes containing the samples were placed into a fridge, at 4°C overnight, to be processed the next day for membrane filtration.

### **Ecological Parameters**

The differences between each stream was assessed using ecological parameters such as stream depth, pH, temperature, conductivity, and alkalinity. The depth of the stream was measured, using a meter stick, at the site of collection and at the deepest region of the stream nearest to the collection site. The conductivity and pH were measured using probes from YSI incorporated, YSI 30 and YSI 60 respectively. Both probes were also used to measure temperature. Conductivity indicates the concentration of dissolved electrolytes such as sodium, nitrate and phosphate (Behr, 2017). Alkalinity was measured using a HACH Test Kit. The kit

measured alkalinity by first adding the provided packet of phenolphthalein indicator to 50 ml of stream water and noting any pink coloration. The pH of all the streams were below 8.3 therefore no coloration was observed. Therefore, the first calculation, variable A, was 0 for each stream. Next, the entire packet of bromocresol green-methyl red indicator was added turning the solution green. Lastly, 1.600 M sulfuric acid was used to titrate the solution until it turned rose pink. Typical titration volumes involved 70-100 digits and were recorded as variable B. Phenolphthalein alkalinity was calculated as  $\text{mg CaCO}_3\text{L}^{-1}$  by multiplying the digits A by 2. Total alkalinity was calculated as  $\text{mg CaCO}_3\text{L}^{-1}$  by adding the digits of A and B and then multiplying by 2.

### **Silver Sampling**

At the point of sample collection, the concentration of silver was tested to understand the native concentration of silver within the streams. For the test, duplicate samples of water (5 mls) were directly collected from the stream with a syringe. Samples were filtered (0.22  $\mu\text{m}$  pore size EZFlow Syringe Filter) into a new plastic tube containing 100  $\mu\text{ls}$  of concentrated nitric acid to achieve a final concentration of 2% nitric acid. To determine if the concentration of silver was affected by our collection methods, a sample of autoclaved ultrapure water (18.2  $\Omega$ ) was brought to the collection site in a one-liter bottle and tested. Duplicate 5 ml samples were obtained from the bottle for silver analysis. A sample was also transferred to a Whirl-Pak bag and processed just like the stream samples. All samples taken at the stream were immediately placed in a mini cooler to limit light exposure, as this can reduce silver ions out of solution.

In order to verify the silver release from the silver- and control-coated coupons, samples were taken at the 0-, 24-, 48-hour time points throughout the incubation. In a laminar flow hood, samples were collected and processed as described for the day of stream sample collection. Duplicate 5 ml aliquots were obtained and filtered into a tube containing 100  $\mu$ ls of concentrated nitric acid. While collecting samples for silver analysis, Petri dishes were removed from the incubator and covered in foil when not in use.

The concentration of silver ions in each silver sample was determined using inductively coupled plasma mass spectroscopy (ICP-MS) (Agilent Technologies 7900). The standards were prepared in acid-washed 50 ml conical tubes. Acid-washing involved spraying 2% ultrapure nitric acid into each tube, ensuring that the inner surface was covered, followed by discarding the acid, and repeating two more times. Seven standards were made using an Agilent Technologies Environmental Calibration Standard (part# 5183-4688) with an initial silver ion concentration of 10 ppm. The calibration standards were diluted to appropriate concentrations of 0.0125, 0.05, 0.1, 5, 100, and 200 ppb using 2% ultrapure nitric acid by weight via a top loader balance. To verify the calibration standards' accuracy, an additional verification standard was prepared, using an Agilent Technologies Initial Verification Standard (part# 5183-4682). The verification was prepared in the same manner to a concentration of 12.5 ppb. After the standards were prepared, they were measured with the ICP-MS to create a calibration curve. The samples were measured and fit inside the standard calibration curve to determine the  $^{107}\text{Ag}^+$  and  $^{109}\text{Ag}^+$  concentrations.

### Culture-Dependent Technique

The procedure concerning bacterial collection was generated from EPA methods 1603: Escherichia coli (E. coli) in Water by Membrane Filtration Using Modified membrane-Thermotolerant Escherichia coli Agar (Modified mTEC) and 9132: TOTAL COLIFORM: MEMBRANE-FILTER TECHNIQUE (United States Environmental Protection Agency, 2002; United States Environmental Protection Agency, 1998). Bacteria were collected from each water sample via the membrane filtration technique. Prior to filtration, the funnel and filtration units were autoclaved. Before the samples were processed, blanks were run on each unit. This consisted of adding 20 mls of sterile PBS into each unit containing a sterile 0.45 µm filter membrane. The filters were removed using sterile forceps and placed on TSA agar plates. After blanks were run, the filtration and funnel units were placed into a sterile cabinet and exposed to ultraviolet light for 15 min to sterilize. Afterwards, samples were processed by stream. The first three manifold slots were used to process the control-treated samples with each being designated to one of the three replicates, the same was done with the last three manifold units and the silver-treated replicates. For each replicate, multiple dilutions were needed and coordinated with the media (TSA, tryptic soy agar; Modified mTec agar, M-Enterococcus agar, and Carbapenem-Resistance Enterobacteriaceae agar) used to analyze the culture-dependent bacterial populations. For each replicate, 10 samples were run. Specifically, dilutions of 1/1000, 1/10000, and 1/100000 were run for TSA and CRE, a 1/10 dilution and undiluted sample were run for Mtec and ME. For each dilution a volume of 20 mls was applied to the filter in order of most dilute to least dilute. The time taken to process all samples for one stream was between 1 hour and 30 minutes and 2 hours, which was longer than the 30 minutes specified by EPA method 9132. Even though the EPA standard time was exceeded, a pre-trial experiment was run to ensure the

sterility of the filtration units during sample processing. The experiment involved filtrating straight stream water samples through the units while running blanks at the beginning, middle, and end of the filtration process. None of the blanks showed growth. Following all the stream samples within a trial, 20 mls of the undiluted ultrapure water samples were passed through filters and plated on TSA.

The bacteria present within each sample was examined by its growth on various agar. Tryptic soy agar (TSA) is an all-purpose agar that allows a wide-range of bacterial species to grow. The remaining three agars were selective for enteric bacteria. Modified mTec agar (mTec) differentiates for *Escherichia coli*, a standard among the scientific community for water-borne bacterium. EPA method 1603, recommends *E. coli* as a measure of fresh, estuarine and marine water quality (U.S. E.P.A., 2002). M-Enterococcus agar (ME) selects specifically for *Enterococcus* and, like *E.coli*, it gave an insight into water quality. To gain a deeper understanding as to the status of antibiotic resistance within the natural aquatic ecosystem, Carbapenem-Resistant Enterobacteriaceae agar (CRE, HardyCHROM™) was used for the selection of *E.coli*, *Klebsiella*, *Enterobacter*, and *Serratia spp.* CRE differentiated the colonies based on the Gram-negative species and their resistance to carbapenems; dark blue colonies represent non-susceptible *Klebsiella*, *Enterobacter*, and *Serratia spp.* (KES) and pink/magenta colonies are presumptively non-susceptible *E.coli*. Clear colonies are carbapenem resistant, unidentified colonies. The CRE agar was covered in aluminum foil and stored in a 4°C fridge because of its light and temperature sensitivity.

Once the agar plates of an entire stream were processed, they were placed in a 37°C incubator for 24 hours, except for mTec. Following EPA method 1603, the mTec plates were incubated at 37°C for 2 hours in a dry incubator followed by 22 hours in a 44.5°C water bath.

When the mTec plates were transferred into the water bath, they were sealed inside of Whirl-Pak bags and weighed down. After the plates were incubated, they were photographed within a laminar flow hood and manually counted using the image processing software ImageJ. Bacterial counts were reported in number of colonies per 1 ml by multiplying the plate count by the dilution factor then dividing by 20 to account for the volume of sample that was filtered (20 mls). Any plate that had below the quantification limit of 30-300 colonies but at least one colony (limit of detection) is considered interval-censored data but was estimated using the same arithmetic. If a set of plated samples were considered too numerous to count (TNTC) (>300 colonies) then it is right-censored data, and, to estimate count, the sample was multiplied by its greatest dilution factor and reported as (>300/dilution factor x 20).

### **Statistical Analysis**

The bacterial counts were compared based on trial and treatment using the statistical software R and packages: ggplot2, dplyr, and Rmisc (Hope, 2013; R, 2018; Wickham, 2016; Wickham et al., 2020). The natural log was taken for the bacterial counts to adjust the variances to create equal variances among treatments and trials. A two-way ANOVA was used to assess the adjusted bacterial counts based on treatment and trial. If an interaction was seen between counts and trial, a Tukey analysis was used to compare the difference between treatments within each trial.



## Chapter 3

### RESULTS

To determine the possible impact of silver ions on bacterial populations within the environment, three streams on the Penn State Behrend campus were chosen to provide a broader perspective in regards to runoff. Reference, upstream of campus within the WinterGreen Gorge, likely received runoff from the nearby Interstate-90 (Figure 3a). GlenHill, located within the center of the campus, would give insight into college campus runoff (Figure 3b). Lastly, TroutRun, at the bottom of the campus near the athletic fields, received runoff from the athletic fields and the highway, Bayfront Connector (Figure 3c).

Ecological parameters, including pH, temperature, conductivity, alkalinity, and depth, varied among the streams and trials, likely, due to the difference in collection times. Considering standard deviation, alkalinity was similar across all streams and trials, with a spike at trial 3 in GlenHill and TroutRun. Conductivity trended downward for Reference, while fluctuating in GlenHill and TroutRun. Conductivity trended downward for Reference, while fluctuating in GlenHill and TroutRun, with GlenHill having the highest levels overall (Table 1). The depth of each stream, where samples were collected, varied slightly by trial. The deepest regions of the streams increased in depth when following rainfall from the previous night, trials 2 and 4 (Table 1). The temperature and pH levels were the most notable ecological changes among the trials (Table 1). The pH trended upward for all streams but never exceeded a pH of 8. As the trials approached fall and winter, the temperature decreased across all of the streams (Table 1).

In order to confirm silver release from silver-coated coupons and determine an approximate concentration of silver in the streams, inductively coupled plasma mass spectroscopy (ICP-MS) was used. During incubation with silver- and control-coated coupons, samples were taken at 0, 24, and 48 hours to be analyzed. The calibration curve plots the seven

standards of trial one and represents that of the remaining three trials (Figure 7). The ultrapure samples collected at the site of each native stream showed silver concentrations below the LOD to 26 ppt. Silver concentrations within the native streams never exceeded 0.5 ppb; GlenHill and TroutRun measured at or below the limit of detection (12.5 parts per trillion (ppt)), while Reference ranged from the LOD (limit of detection) to 0.480 ppb (data not shown). The silver concentrations measured from the incubated samples were averaged for all four trials, including their three replicates, for each stream (Figure 8). The silver incubated samples demonstrated an, overall, increase in silver concentration over the 48 hours of incubation, while the incubated control samples showed concentration at or near the LOD (never exceeding 0.448 ppb) (Figure 8). The silver incubated ultrapure water samples showed a greater release of silver ion than those of the stream samples, while the incubated control samples showed concentrations at or near the LOD (never exceeding 0.995 ppb) (Figure 8). The reduction in silver ion concentration in stream samples could have been due to silver complexation with other ions present.

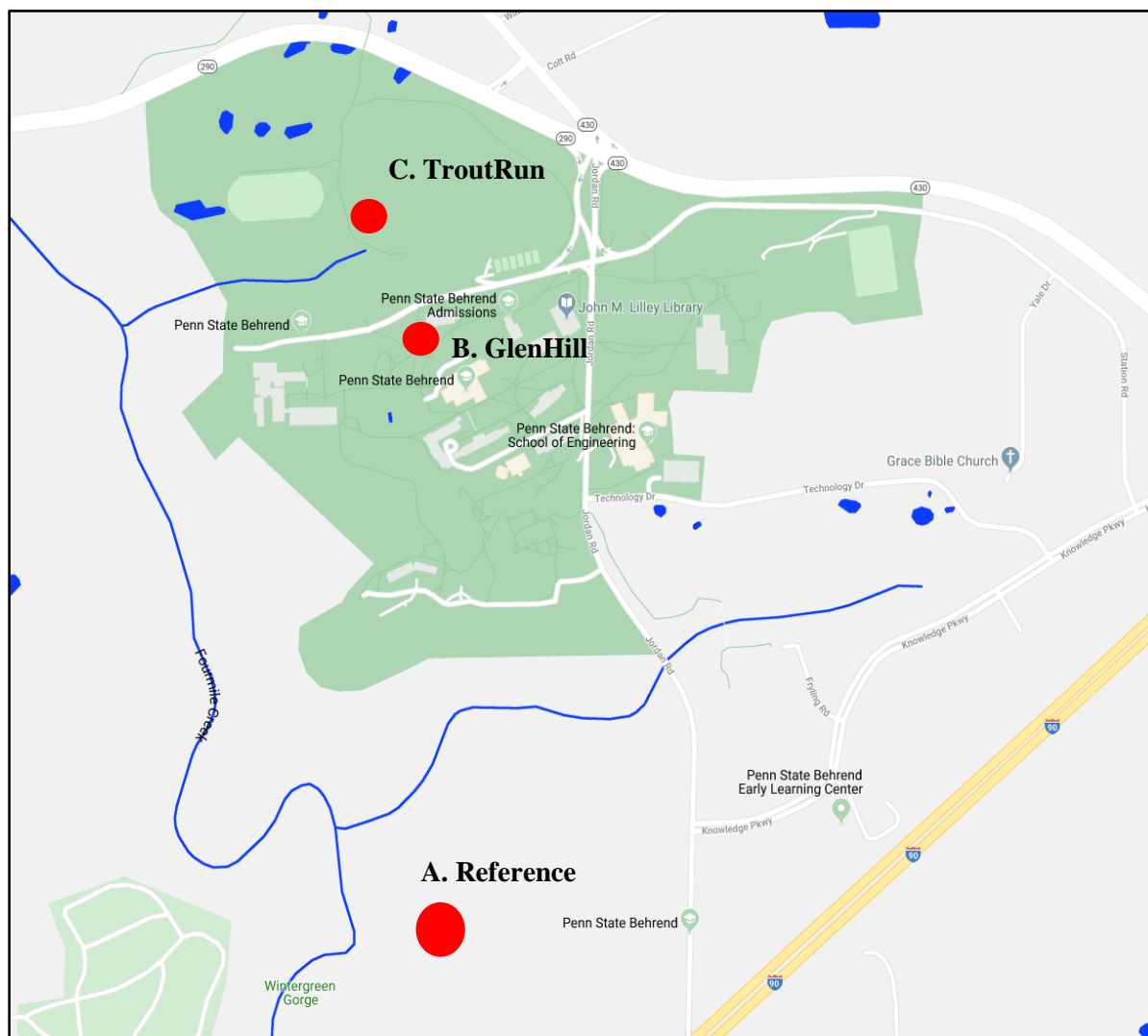
For bacterial analysis, samples of each stream were collected in triplicate. Each of these replicates were divided equally into two treatments, exposure to a silver- or control-coated coupon. After the samples were incubated with their coupon for 48 hours, they were serially diluted. The culture-dependent approach involved filtering these water samples and allowing growth on four different agar plates for 24 hours. The plates were counted and adjusted based on their dilutions to give bacterial counts per 1 ml. Any plates with a colony count below the limit of quantification (30-300 colony forming units (CFUs)), but above the limit of detection (1 CFU), were marked interval-censored, but still adjusted based on dilutions. The interval-censored counts were due to, either, over diluting the samples and/or samples being plated at their highest concentration, but still arriving at a total of <30 CFUs. Plates above the limit of

quantification (>300 CFU) were considered too numerous to count (TNTC) and marked right-censored. These were estimated to be 300 CFUs and then adjusted based on dilution. The right-censored plates arose from not sufficiently diluting the samples. Samples within a trial could not be run/diluted again due to the absence of additional water samples.

In an attempt to create equal variances between the triplicate samples given orders of magnitude differences between the control and silver treatment, a log transformation was used to present all the bacterial counts. The samples, within an agar, were then analyzed using a two-way ANOVA test to determine the interaction between trial and treatment. This test showed that for all streams, each agar had a significant interaction between the bacterial counts and trial (Figures 4-6). The interactions between counts and trials could be reflected as a “date effect”, that is, an effect due to the differences in ecological parameters between trials (Tables 1). Temperature may have been an important factor as it declined with each trial and bacteria are not capable of thermoregulation (Panoff et al., 1998).

In order to analyze silver’s effects directly, a TukeyHSD comparison test was used on the triplicate pairs for each trial. The Tukey test was able to determine whether silver was having a significant effect on the counts independent of the trial effects. Within all streams, the samples exposed to silver exhibited a significant decrease in bacterial growth compared to the control treatment. However, two agars, Mtec and ME, had exceptions within specific trials of each stream (Figures 4-6). These exceptions did not show any significant effect between the silver and control treatments. Excluding the exceptions, silver had a significant effect on all sample pairs (control vs silver treatment) but, due to the trials’ “date effect”, silver was observed not to have a consistent effect.

Unlike the other agars, CRE agar differentiated colonies based on carbapenem susceptibility. The blue colonies represented carbapenem non-susceptible *Klebsiella*, *Enterobacter*, and/or *Serratia spp.* (KES), while pink to magenta denoted non-susceptible *E. coli*, and clear colonies were non-susceptible, unidentified species. Of the colonies observed, no non-susceptible *E. coli* were found, but rather a varying ratio between clear and blue colonies. In the first trial each stream only consisted of a few (1-2) blue colonies while the majority was clear. The second trial showed GlenHill and TroutRun to have a 1:1 ratio of blue to clear colonies, while reference still had only a few blue colonies. The third trial had Reference and TroutRun with few blue, but GlenHill still had 1:1 blue to clear colonies. For all three streams, the fourth trial had a majority of blue colonies.



**Figure 3: Map of Penn State Behrend Campus with Labeled Collection Sites**

Reference (A) is located upstream of the Penn State Behrend Campus and receives runoff from Interstate-90 (42.108715, -79.984119). GlenHill (B) is near the Turnbull Hall, the psychology building on the Penn State Behrend Campus, and receives campus runoff (42.121948, -79.985848). TroutRun (C) is at the bottom of campus, beneath the athletic bridge leading up to Behrend fields, and receives runoff from the athletic fields and Bayfront Connector (42.120191, -79.984927).

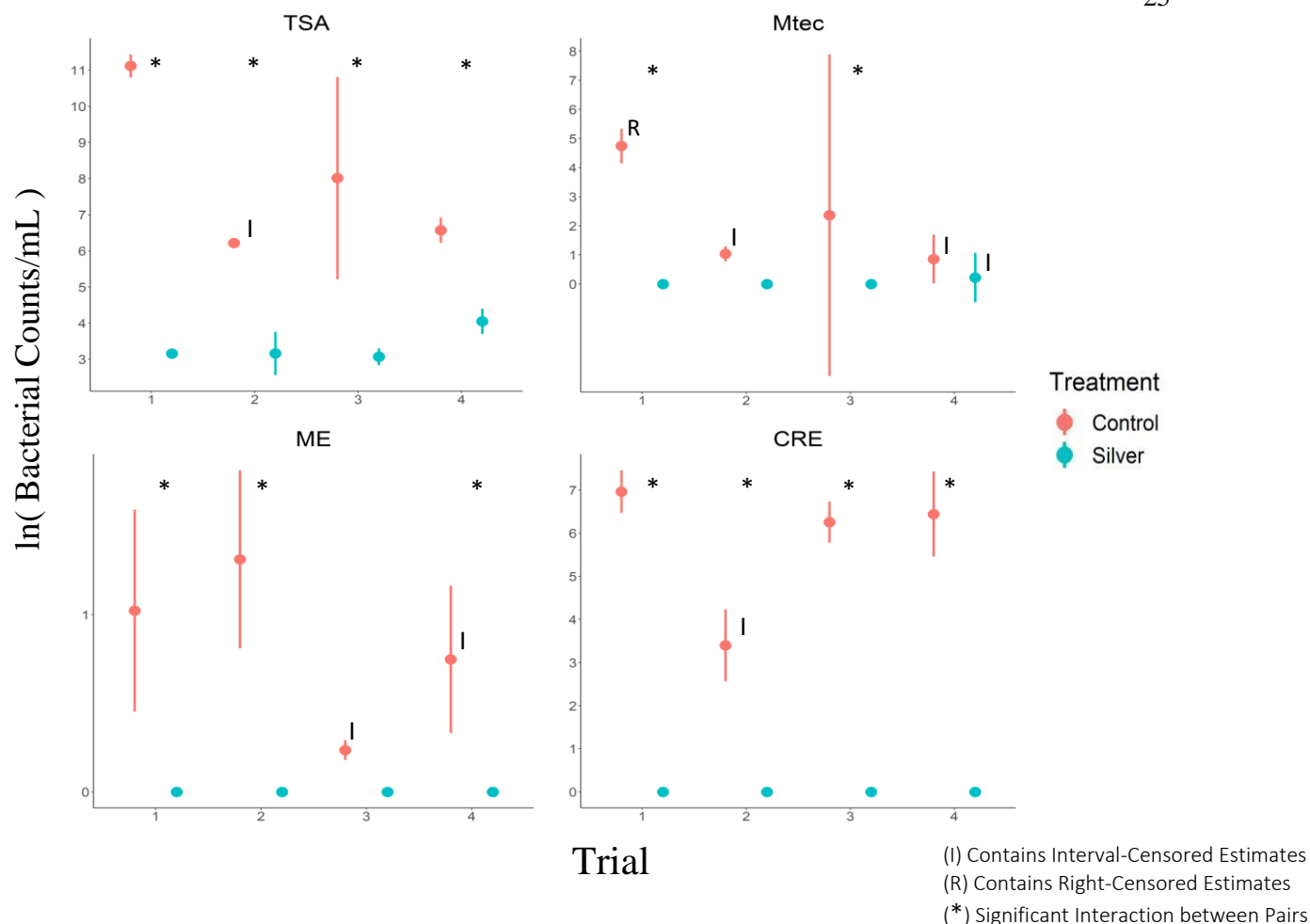
**Table 1: Ecological Parameters of each Stream for each Trial**

The first trial was collected in August, followed by two in October, and one in November. A start time was recorded before a stream was processed. Temperature was measured by both the pH and conductivity probes and then averaged. Temperature values reported with no standard deviations signify the temperature readings were the same across both probes. Alkalinity was measured in triplicate and averaged. Trials 2 and 4 experienced rainfall the previous nights. (A) Reference, located upstream of campus, had an observable higher flow during trial 4. Temperature of each stream decreased by trial. The overall trend for pH was to increase by trial.

A Reference				
Parameter/Trial:	Trial 1	Trial 2	Trial 3	Trial 4
Date of Collection	8/12/2019	10/7/2019	10/21/2019	11/4/2019
Time of Collection	8:50 AM	9:05 AM	8:34 AM	8:24 AM
Temperature (°C)	17.6 ± 0.3	13.0	9.6	5.3
pH	6.57	7.41	7.37	7.48
Conductivity (µS)	937	866	852	278
Alkalinity (mg CaCO <sub>3</sub> /L)	130 ± 30	120 ± 20	120 ± 20	68 ± 5
Depth of Stream Collection Site (cm)	14.5	12.0	9.5	10.9
Depth of Deepest Section of Stream (cm)	14.5	20.0	17.7	22.5

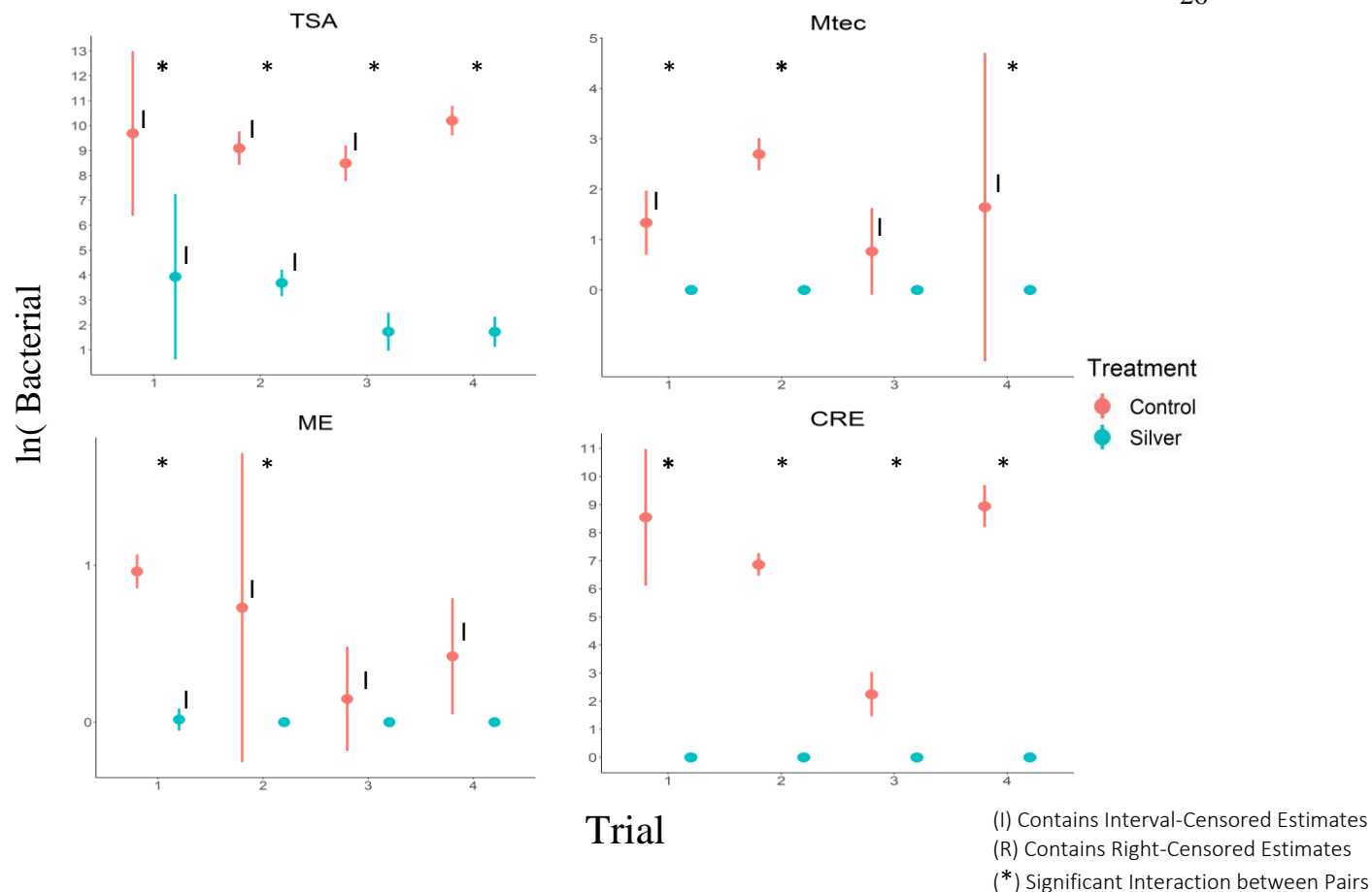
B GlenHill				
Parameter/Trial:	Trial 1	Trial 2	Trial 3	Trial 4
Date of Collection	8/12/2019	10/7/2019	10/21/2019	11/4/2019
Time of Collection	10:20 AM	7:25 AM	7:13 AM	7:30 AM
Temperature (°C)	18.7	14.75 ± 0.07	11.35 ± 0.07	9.0 ± 0.2
pH	6.60	7.63	7.71	7.71
Conductivity (µS)	2732	850	2568	1517
Alkalinity (mg CaCO <sub>3</sub> /L)	150 ± 40	140 ± 60	210 ± 40	150 ± 20
Depth of Stream Collection Site (cm)	16.00	11.8	17.5	18.5
Depth of Deepest Section of Stream (cm)	29.5	40.0	31.8	39.5

C TroutRun				
Parameter/Trial:	Trial 1	Trial 2	Trial 3	Trial 4
Date of Collection	8/12/2019	10/7/2019	10/21/2019	11/4/2019
Time of Collection	9:45 AM	8:03 AM	7:53 AM	7:53 AM
Temperature (°C)	16.8	14.4	11.6	8.1
pH	7.74	7.83	7.93	7.98
Conductivity (µS)	1033	726	945	839
Alkalinity (mg CaCO <sub>3</sub> /L)	200 ± 30	190 ± 30	240 ± 30	189 ± 8
Depth of Stream Collection Site (cm)	7.0	9.9	7.3	13.4
Depth of Deepest Section of Stream (cm)	7.0	15.5	18.1	18.8



**Figure 4: Bacterial Counts for the Reference Stream for each Agar by Trial.**

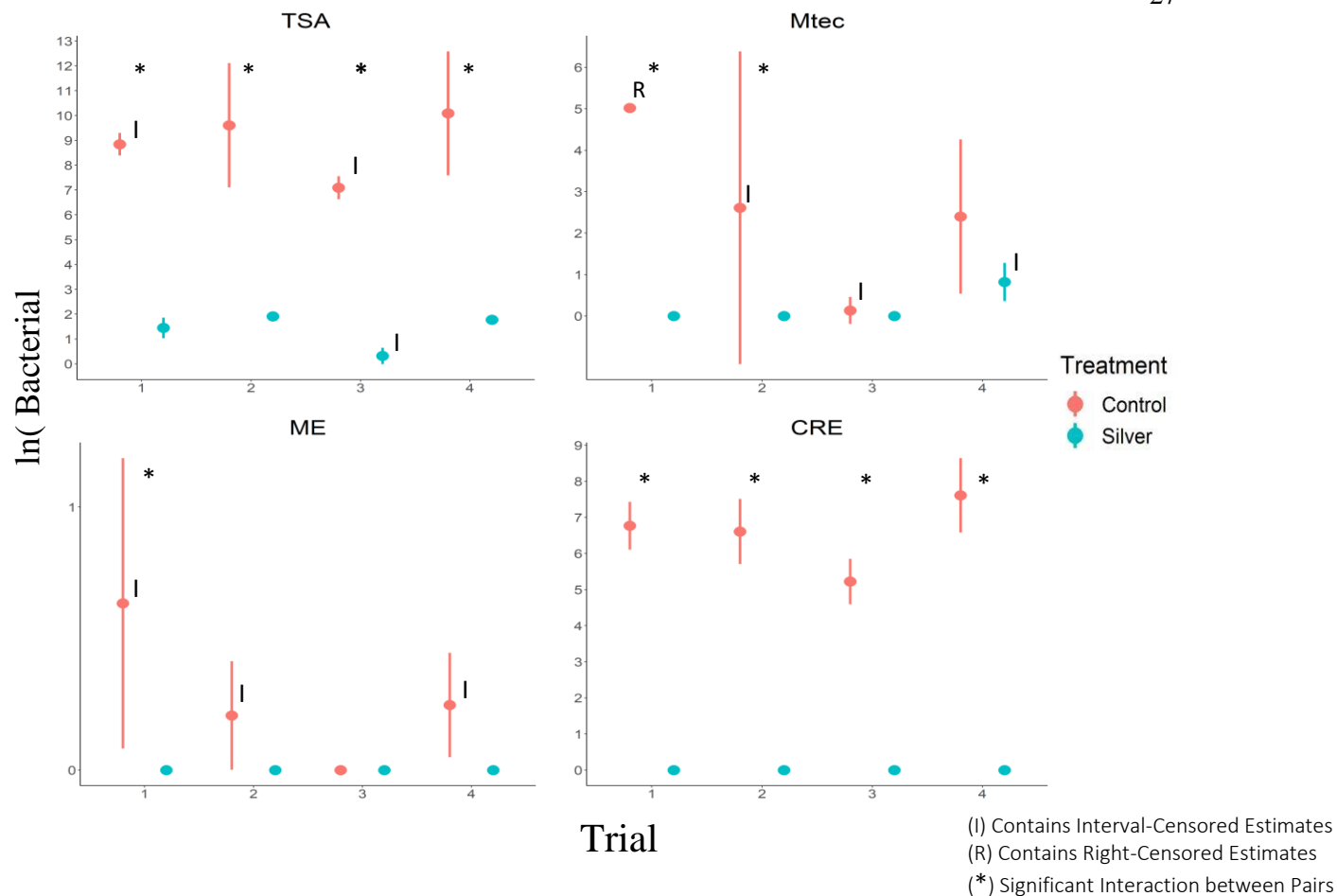
Samples of each trial were collected in triplicate, split equally into either a silver or control treatment, incubated, filtered, then counted for colony forming units. Each point on the figure represents the natural log of bacterial counts for triplicate samples. The bars on each point indicate the 95% confidence interval. The two-way ANOVA test showed a significant interaction between the natural log of bacterial counts and trial for each agar ( $p_{TSA} = 1.84E-07$ ,  $p_{Mtec} = 3.41E-03$ ,  $p_{ME} = 9.51E-06$ ,  $p_{CRE} = 3.51E-10$ ). A Tukey comparison test was run on the ANOVA test data above and showed the silver treatment to have a significant effect on bacterial growth on all sample pairs (control and silver treatment) except for trials 2 and 4 of the Mtec agar ( $p_2 = 0.760$ ,  $p_4 = 0.974$ ) and trial 3 of the ME agar ( $p = 0.330$ ). Therefore, silver had a significant effect on all but three sample pairs, but silver did not have a consistent effect across trials.



**Figure 5: Bacterial Counts for the GlenHill Stream for each Agar by Trial**

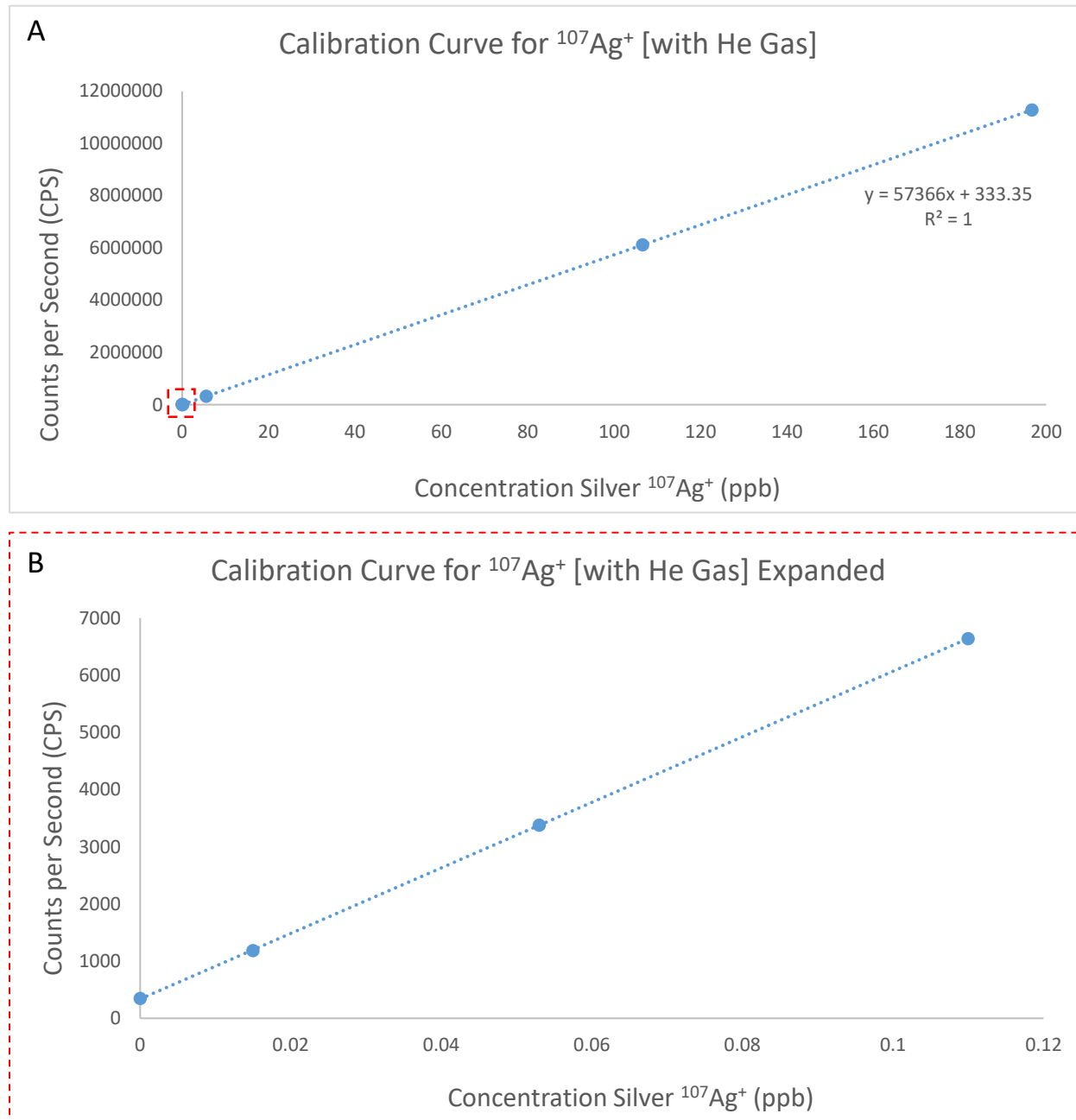
Samples of each trial were collected in triplicate, split equally into either a silver or control treatment, incubated, filtered, then counted for colony forming units. Each point on the figure represents the natural log of bacterial counts for triplicate samples. The bars on each point indicate the 95% confidence interval. The two-way ANOVA test showed a significant interaction between the natural log of bacterial counts and trial for each agar ( $p_{\text{TSA}} = 4.64\text{E-}01$ ,  $p_{\text{Mtec}} = 0.0168$ ,  $p_{\text{ME}} = 1.94\text{E-}05$ ,  $p_{\text{CRE}} = 1.92\text{E-}10$ ). A Tukey comparison test showed the silver treatment to have a significant effect on bacterial growth on all sample pairs (control and silver treatment) except for trial 3 of the Mtec agar ( $p_3 = 0.499$ ) and trials 3 and 4 of the ME agar ( $p_3 = 0.936$ ,  $p_4 = 0.074$ ). Therefore, silver had a significant effect on all but three sample pairs, but silver did not have a consistent effect across trials.





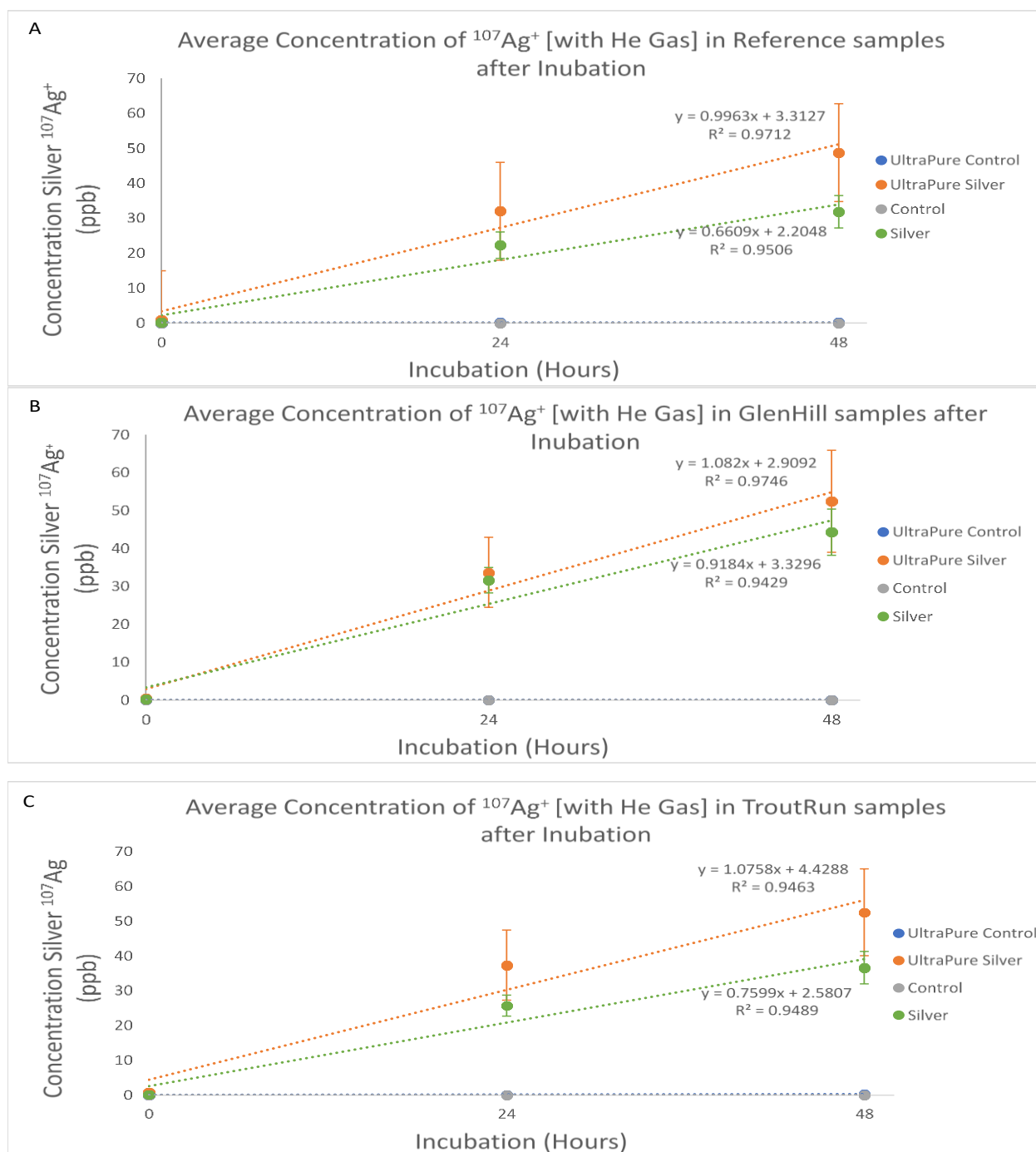
**Figure 6: Bacterial Counts for the TroutRun Stream for each Agar by Trial**

Samples of each trial were collected in triplicate, split equally into either a silver or control treatment, incubated, filtered, then counted for colony forming units. Each point on the figure represents the natural log of bacterial counts for triplicate samples. The bars on each point indicate the 95% confidence interval. The two-way ANOVA test showed a significant interaction between the natural log of bacterial counts and trial for each agar ( $p_{TSA} = 4.91E-06$ ,  $p_{Mtec} = 3.41E-05$ ,  $p_{ME} = 1.25E-04$ ,  $p_{CRE} = 1.78E-06$ ). A Tukey comparison test showed the silver treatment to have a significant effect on bacterial growth on all sample pairs (control and silver treatment) except for trials 3 and 4 of the Mtec agar ( $p_3 = 1.00$ ,  $p_4 = 0.081$ ) and trials 2,3, and 4 of the ME agar ( $p_2 = 0.145$ ,  $p_3 = 1.00$ ,  $p_4 = 0.0538$ ). Therefore, silver had a significant effect on all but five sample pairs, but silver did not have a consistent effect across trials.



**Figure 7: Representative ICP-MS Calibration Curve towards  $^{107}\text{Ag}^+$  using He Collision Mode**

(A) The calibration curve of the seven standards made for Trial 1 silver analysis ( $R^2 = 1$ ). The  $R^2$  values for the other three trials were also equal to 1. Measurements were made using inductively coupled plasma mass spectroscopy (ICP-MS). (B) An expanded view of (A) to show the lower concentration standards not depicted in (A).



**Figure 8: Average Concentration of  $^{107}\text{Ag}^+$  [with He Gas] for each Stream**

Average concentration of silver ion ( $^{107}\text{Ag}^+$ ) for all four trials and their 3 replicates,  $n=12$ , during 48 hours of incubation with silver or control treatment measured using ICP-MS with He collision mode. Samples were taken at 0, 24, and 48 hours of incubation. Error bars represent standard error of the mean. All three streams showed the ultrapure silver samples to have a higher overall silver concentration compared to the stream silver samples. All control treatments had a concentration near or below the limit of detection (12.5 ppt).

## Chapter 4

### DISCUSSION

Collected stream samples were incubated for 48 hours with either a silver- or control-coated coupon. Silver analysis with the ICP-MS showed that silver ions were released into each silver treated water sample, while the control samples showed concentrations at or near the limit of detection (12.5 ppt) (Figure 8). Therefore, within a trial, any effect seen on bacterial growth was due to the presence of silver ion.

Silver's effect could not be viewed for trials collectively because a two-way ANOVA test showed an interaction between bacterial counts and the trials (Figure 5). The trials were separated by weeks, allowing the streams to exhibit different ecological parameters that could have affected the streams' ecosystems and bacterial composition/abundance, a "date effect" (Table 1).

The effects of silver ions are best observed through individual trials because they are independent of the "date effect". Silver showed a significant decrease in bacterial growth with nearly all silver exposed samples, but had exceptions with the Mtec and ME agars (Figures 4 -6). It should be noted that these two agars exhibited relatively low bacterial counts throughout all trials; the median counts for each were 3.40 and 0.550 CFUs per ml, respectively. While, TSA and CRE maintained high bacterial populations; their median counts were 6750 and 797.5 CFUs per ml, respectively. One possible explanation for these lower values could be the temperature of the streams (Table 1). The bacteria grown on the Mtec and ME agars, *E. coli* and enterococcus, are mesophilic and therefore have growth ranges between 21-49 °C and 10-45 °C, respectively

(Ramsey et al., 2014; Strocchi et al., 2006). On all trial dates, the temperatures of the streams were at the low end or below these ranges.

The use of different agars allowed for insight into the bacterial composition of each stream. The ME agar selected for the Gram-positive bacterial genus enterococcus, Mtec and CRE agars selected for Gram-negative species, and TSA, an all-purpose agar, allowed for the growth of any culturable bacteria. The species selected by the Mtec and ME agars, *E. coli* and enterococcus respectively, were incorporated into the study because of their persistence within the environment and incorporation in water testing standards such as fecal pollution indication (Boehm & Sassoubre, 2014; Byappanahalli et al., 2012). The *E. coli* colonies showed susceptibility to the silver treatment as no CFUs were present for the first three trials in any stream, with two exceptions. However, unusual growth was found on Mtec agar in trial 4 of Reference and TroutRun, indicating silver resistant colonies (Figures 4 and 6). Similarly, enterococcus had no CFUs when exposed to the silver treatment, except for trial 1 at the GlenHill stream (Figure 5). Overall, very little growth was observed on the selective agars derived from the samples that were incubated with silver-coated coupons.

The CRE agar encompassed a wider range of Gram-negative bacterial species, but also differentiated for resistance to the carbapenem class of antibiotics. Of the colonies observed, no non-susceptible *E. coli* were found, denoted by a pink to magenta colony. However, the dilutions used for CRE were higher than that of Mtec, therefore, their presence may have been out of our scope of dilutions. The CRE plates consisted of a varying ratio between clear and blue colonies. The blue colonies represented carbapenem resistant KES (*Klebsiella aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Enterobacter cloacae* complex, and *Serratia marcescens*) while the clear colonies were resistant, but unidentified, bacterial species. With each trial, the ratio

between blue and clear colonies changed. Starting out with very few blue colonies in the first trial, but trial 4 had a majority of blue colonies. This result may be due to the, as-mentioned-above, “date effect” where the differing ecological parameters create changing selective pressures for particular bacterial species to grow over others. In this case, carbapenem resistance KES are favored over others during the later trials with lower temperatures and higher pH levels (Table 1). Importantly, all silver treated CRE samples derived from silver-coated coupons showed no growth, indicating their susceptibility to silver ions (Figures 4-6).

Carbapenems encompasses the widest spectrum of activity and greatest potency against Gram-negative and Gram-positive bacteria (Papp-Wallace et al., 2011). They are primarily used as “antibiotics of last resort”, that is, when all other antibiotics are ineffective, whether because of insufficient potency or resistance, carbapenems can save the lives of these helpless patients (Papp-Wallace et al., 2011). However, the recent emergence of multidrug-resistant pathogens has threatened the effectiveness and accountability of this important class of drugs (Papp-Wallace et al., 2011). The counts observed from the CRE agar derived from control-coated coupons confirms the presence of carbapenem resistance in our environment. Luckily, our data shows that silver is an effective antibacterial agent against these resistant species.

TSA exhibited the overall highest bacterial counts, as would be expected from its all-purpose/inclusive nature for culturable bacteria (Figures 4-6). Even though there was a significant decrease in silver treated samples, a large amount of growth was still observed on the plates. Their bacterial counts were the highest compared to the other three agars; the median counts, for all three streams, was 8.50 CFUs per ml. This indicates that there are thriving silver resistant species within all three streams. The colonies that make up the silver treated samples on TSA could be either Gram-positive or Gram-negative species, which weren’t selected for on any

of the other agars used. A few studies have shown that the larger cell wall of Gram-positive organisms may be responsible for their innate resistance (Feng, et al. 2000; Kawahara et al. 2000). The bacterial cell wall is composed of a mesh-like structure of peptidoglycan, and its thickness could provide protection to the cell from silver ion entering the cytoplasm (Feng, et al. 2000).

Since Gram-negative bacteria lack a large cell wall, resistance is thought to be acquired through mutation (endogenous) or horizontal transfer (exogeneous) (Randall et al., 2015). In order to prevent silver from entering the Gram-negative cell's periplasm, endogenous mutations can occur in the transcription factor responsible for the expression of OmpC and OmpF porins, thereby reducing the membrane permeability to silver (Randall et al., 2015). Exogenous silver resistance has been observed via proteins coded from a silver resistance gene cluster found within bacterial plasmids (Mijnendonckx et al., 2013; Silver et al., 2003). The plasmid pMG101 of *Salmonella enterica* serovar Typhimurium was the first to be characterized, and it was found to grant resistance to silver, mercury, tellurite as well as several antibiotics (Mijnendonckx et al., 2013; Silver et al., 2003). The silver resistance region of the plasmid contains a cluster of 9 genes, 8 of which have been observed with other known metal resistance determinants (Silver et al., 2003; Silver et al. 2006). The gene cluster responsible for silver resistance is highly conserved on several plasmids belonging to the incHI-2 incompatibility group of various *Salmonella* serovars and plasmids of *Serratia marcescens* (Mijnendonckx et al., 2013). The resistance mechanism relies on, SilP, an ATPase efflux pump, which transports the silver ions from the bacterium's cytoplasm to the periplasm (Mijnendonckx et al., 2013; Randall et al., 2015). SilF, a periplasmic protein, hypothesized to act as a chaperone and transport silver ions from SilP to the SilCBA complex (Mijnendonckx et al., 2013; Randall et al., 2015). Another

periplasmic protein, coded by the *silE* gene, was found to bind up to 38 silver ions and has been hypothesized to be a first line of defense and sequester silver ions (Mijnendonckx et al., 2013; Silver et al., 2003; Randall et al., 2015). The SilCBA complex is formed from three polypeptides: an outer membrane factor (*silC*), a membrane fusion protein (*silB*), and an efflux pump (*silA*) (Silver et al., 2003; Franke, 2007; Randall et al., 2015). The complex acts as a membrane-potential dependent cation/proton antiporter system spanning the entire cell membrane to act as a heavy metal efflux pump (Silver et al., 2003). With enough environmental selective pressure, silver resistance could spread.

Overall, the majority of the trials showed silver to be an effective antibacterial agent against the cultured bacterial populations. Specifically, it was capable of killing off the carbapenem resistant populations, an important, “last-resort” antibiotic. Given these results it would be interesting to determine silver’s effectiveness against other antibiotic resistant populations. Silver, however, was not able to inhibit all culturable bacterial growth as growth was still observed on TSA agar. The silver resistant populations that make up the TSA agar could be either Gram-negative or Gram-positive bacteria with either exogenous or endogenous mutations allowing for their survival. Depending on how the majority of silver resistance is occurring, whether through endogenous mutations or horizontal transfer, will have important implications for the continued effectiveness/usefulness of silver against bacterial populations. A culture-independent study was being done to provide a broader understanding of changes within the bacterial populations, but could not be concluded due to the current pandemic that has stopped all on-site activities on our campus. These studies are important to continue to preserve the power of silver and keep our environment safe.



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## ACADEMIC VITA

# Tyler Hostetler

### Education

The Pennsylvania State University, Erie, PA

May 2020

- Bachelor of Science in Biology
- Minor in Chemistry
- Schreyer Honors Program
- 2017 Penn State Behrend CRC Outstanding Freshman in Chemistry Award
  - Scored 98<sup>th</sup> percentile in the nation for the ACS general chemistry exam

### Research Experience

*A Culture-dependent and Culture-independent approach to Examine the Effects of*

*Silver Ions on Bacterial Compositions within Local Streams* **September 2017- Present**

- Collaborate with Dr. Beth Potter
- 22<sup>nd</sup> Annual Environmental Chemistry and Microbiology Student Symposium
  - Placed 3<sup>rd</sup> for poster presentation
- 15<sup>th</sup> Annual Regional Science Consortium Research Symposium
  - Placed 1<sup>st</sup> for oral presentation

### Academic Experience

**Physical Chemistry: Quantum Chemistry**

**Spring 2019**

- Quantum chemistry and atomic and molecular spectroscopy
- Chemical Kinetics involving rate laws, collision theory, and activated complex theory

**Immunology**

**Spring 2019**

- Antigen recognition and innate immune responses to the development of acquired immunity
- Development, selection, and survival of B and T lymphocytes

### Campus Involvement

- **Biology Club, Member** **September 2016 - Present**
  - Attend talks on current research projects occurring at Penn State Behrend
  - Meet with students and professions in the field to learn more about career options
- **Jazz Band, Alto and Tenor Saxophone** **September 2007 - Present**
  - Lead Alto/Tenor in college Jazz band
  - Lead Alto in High school Concert, Jazz, and Marching band

## **Work Experience**

- **Server/Bartender, Marriott**
- **Sales Consultant, Best Buy**
- **Sales Consultant, Office Max**

**May 2018 - Present**  
**November 2015 – March 2018**  
**February 2015 – November 2015**

## **Skills**

- **Software: Microsoft Office Suite, R, C++**
- **Hardware: Assembled Desktop Computers**
- **Lab Techniques: Tissue Culture, SDS-PAGE, Western Blotting, PCR, Gel Electrophoresis**