

**Improving the MadiDrop+ Tablet (Point of Use Water Technology) With the Application
of Silver and Copper**

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On my honor as a University Student, I have neither given nor received unauthorized aid on this
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Abstract

The MadiDrop+ tablet is a point-of-use water treatment (POUWT) technology that releases silver microparticles into water for disinfection of bacteria and pathogens. The microporous ceramic tablet has the capacity to treat contaminated water daily for a year, requiring a contact time of 8 hours in 10 liters of water before consumption.⁵ This research examined the potential of adding copper alongside the silver-embedded MadiDrop+ tablet for additional pathogenic disinfection effects, as well as the use of silver and copper as disinfectants for mosquito larvae. Using copper mesh alongside the MadiDrop+ allowed for successful releases of both copper and silver levels. Silver released from the MadiDop+ was successful at lowering mosquito larvae survival rate in water. Similarly, copper was successful in lowering the larvae survival rates. Our results show that increasing the concentration of copper effectively decreases the rates of mosquito larvae survival. Generally, combining silver and copper did not increase the overall larvicidal effectiveness, compared to silver and copper alone. If future findings conclude that copper and silver have synergistic effects on bacterial, mosquito larval, or other pathogenic disinfection, the best method for copper and silver release would be to include 10g fine copper mesh alongside the MadiDop+. Water insecurity is a global issue that can be ameliorated using point of use water treatments; the MadiDrop+ has proven to be successful in both disinfecting water and inhibiting vector carrying mosquitoes from successfully reproducing in drinking water. While copper alone is effective in decreasing survival rates of mosquito larvae, the degree of additional effectiveness that copper and silver together provide is inconclusive, requiring further research.

Introduction

In 2020, about 1 in 4 people around the world lack safely managed drinking water in their homes.¹ This water crisis is exacerbated by climate change, pollution, and increasing water consumption.² Drinking contaminated water can transmit a variety of waterborne diseases, including cholera, dysentery, hepatitis A, and typhoid.³ A typical symptom of these diseases is diarrhea, causing almost 500,000 deaths each year.⁴ Additionally, mosquitoes pose a serious

threat to human health; mosquitoes can carry many diseases that can be transmitted to humans through a mosquito bite, including the Zika virus and malaria; some of these diseases can cause sickness and even death.⁸ Mosquito larvae grow in water, eventually emerging as mosquitoes. To combat instances of waterborne and mosquito-borne diseases, point-of-use water treatment (POUWT) technologies have been employed at the household level to reduce the pathogen load in drinking water. POUWT occurs after water has been collected but before consumption.

At the University of Virginia, the MadiDrop+ was created to effectively disinfect drinking water from pathogenic organisms. The MadiDrop+ is a silver-embedded ceramic tablet.⁵ When placed into 10-20 liters of water with 8 hours of contact time, the tablet gradually releases silver ions for a 3-4 log reduction of coliform bacteria.⁶ Compared to other traditional, single use, and long term use POUWT options, the MadiDrop+ is easy to use, provides residual disinfection, has a perpetual shelf life and can be used daily for a year, and is very low cost.⁵ For these reasons, the MadiDrop+ is seen as a highly effective and socially acceptable technology for water disinfection.

Currently, the MadiDrop+ is highly effective in killing waterborne bacterial pathogens and moderately effective to fight against pathogenic viruses, protozoa, and mosquito larvae.⁵ Copper has the potential to interact with silver and increase the effectiveness of water disinfection across a variety of pathogenic organisms. Previous studies have suggested that copper and silver together have synergistic effects, such that their combined effectiveness of disinfection is greater than the sum of their individual effects.⁷ Therefore, this research aims to analyze the benefits of copper in combination with silver, and its ability to disinfect contaminated water and kill mosquito larvae.

This research can be viewed in two parts: methods of implementing copper alongside the silver MadiDrop+ for adequate release of both ions, and the effectiveness of copper and silver in killing mosquito larvae. Because silver and copper have the potential to act synergistically for water treatment, our research group studied various configurations of copper mesh and a MadiDrop+. The goal of this research project was to determine the most effective arrangement in releasing silver and copper into 10 liters of water over 24 hour time intervals. Additionally, we examined the impacts of silver and copper on mosquito larvae die-off to understand the potential benefits of using these chemicals, both separately and in combination. This research aims to

provide insight on methods and technologies that can effectively disinfect drinking water and terminate mosquito larvae, providing safer water and environments for people around the world.

Materials and Methods

MadiDrop+

Setup

The MadiDrops+ used in this report were fabricated by Silivhere Technologies, Inc in Charlottesville, VA. Our experiments with the MadiDrop+ utilized various configurations of the copper and MadiDrop+ in a total of 16 buckets of 10L deionized (DI) water. The configurations are shown below in Table 1.

Table 1: Configurations of MadiDrop+ and Copper in 10L DI Water

Type of Mesh/MadiDrop+	Mass of Mesh	Configuration
Coarse Copper Mesh	10g	Copper Mesh and MadiDrop+, separated
	10g	Copper Mesh, balled up
	10g	Copper Mesh wrapped around MadiDrop+
Fine Copper Mesh	5g	Fine Mesh
	10g	Fine Mesh
	10g	Fine Mesh and MadiDrop+, separated
MadiDrop+ Alone	–	MadiDrop+

All configurations were duplicated, with the exception of the Fine Mesh (10g) and MadiDrop+ separated, and the MadiDrop+ alone, which were performed in triplicates. These configurations were each placed in separate buckets. Six buckets were set up and sampled from at a time. The coarse copper mesh was sourced from GeBot. The fine copper mesh sheets were sourced from TWP Inc. The estimated dimensions of the 5g fine copper sheets were 5.6in by

5.3in. The estimated dimensions of the 10g fine copper sheets was 11.1in by 5.3in. Dimensions of the coarse mesh could not be determined, therefore the weight was the primary consideration when setting up and comparing the different configurations of mesh. The weight of the mesh was determined using a mass scale.

Sampling

The buckets were sampled every 24 hours to simulate daily use. To collect a sample, a sterile pipette tip was used to mix the water for 10 seconds to allow the copper and/or silver to evenly mix throughout the solution. 20mL of the water was collected in a sterile centrifuge tube. The sample was then acidified with 571 μ L of nitric acid (HNO₃) to achieve a final concentration of 2% by volume. The following equation was used to achieve this HNO₃ concentration.

$$\frac{20\text{mL sample} \times 2}{70} \times 1000 = 571\mu\text{L HNO}_3$$

After sampling, each bucket was emptied and refilled with 10L of DI water and sampled again the following 24 hours. Sampling ended after considerable data was collected and proved to be beneficial for our study.

Measurement

Each sample collected was measured for its levels of copper and silver using a PerkinElmer HGA 900 (Waltham, MA) graphite furnace atomic absorption spectrometer (GFAA). To use the GFAA, the samples were diluted in a 1:1 ratio of 600 μ L sample to 600 μ L of 2% HNO₃, or in a 1:4 ratio of 300 μ L sample to 900 μ L 2% HNO₃. The desired concentration of copper release from these experiments was 600ppb copper (Cu) and below 100ppb silver (Ag) release. These concentrations were desired as they were below the Maximum Contaminant Level for silver, which is 0.1ppm, and copper, which is 1.3ppm.^{10, 11}

Mosquitoes

The mosquito species used in this study were between 1st and 4th instar *Aedes aegypti* mosquitoes. The experiments conducted primarily focused on the evaluation of chemicals at

varying concentrations below the drinking water quality standard, where the Maximum Contaminant Level for silver is 0.1ppm and copper is 1.3ppm.^{10, 11}

Culturing Mosquitoes

The mosquito eggs were sourced from Benzon Research, Inc. The eggs were cultured on a 12 hr day/12 hr night cycle using a lamp timer in an incubator set to 28°C (82.4°F) and 70-85% relative humidity (RH). The larvae were fed daily with ground larval food (mixture of Liver Powder (LP) and Brewer's Yeast (BY)).

Copper and Silver Concentrations

To create the concentration of silver required for the experiments, first a serial dilution of Artcraft Chemicals (CAS No. 7761-88-8) Silver Nitrate (AgNO_3) was used to create a 900ppb stock solution. 8.89mL of this solution was then used to create 40ppb concentrations of AgNO_3 for the experiments in DI water. A serial dilution of Alfa Aesar (CAS No. 7758-99-8) Copper Sulfate (CuSO_4) was used to create a 520ppm stock solution. Table 2 shows how the desired copper test concentrations for experiments were created using the 520ppm stock solution.

Table 2: Creation of Copper Test Concentrations

Desired Concentration	DI Water	Amount of Stock Solution Used
300ppb CuSO_4	199.89mL	0.115mL
600ppb CuSO_4	199.77mL	0.23mL
1200ppb CuSO_4	199.54mL	0.46mL

Setup and Experiment

The concentrations of copper and those of silver were used for the set up of experiments shown in Table 3.

Table 3: Copper and Silver Experiments with Mosquitoes

Instar	Concentrations Tested
Late 3rd and early 4th instar	Controls (0ppb Cu) 300ppb Cu 600ppb Cu 1200ppb Cu
Late 1st and early 2nd instar	Controls (0ppb Cu) 600ppb Cu 40ppb Ag 600ppb Cu and 40ppb Ag

When the desired instar was reached, 25 larvae were transferred into a 250mL beaker containing the test concentrations outlined in Table 2 and placed in the incubator. The larvae were then fed for four consecutive days after being transferred into the beaker, and then fed every other day. The observation of the mosquitoes began as soon as larvae were placed into the Ag/Cu solutions. Every 24 hours, observations of larval mortality, pupae, and mosquito emergence were recorded. When all larvae died or emerged as adults, this observational period ended, and no further recording was necessary.

If the adult emergence in the controls was less than 80%, the test was no longer valid and was terminated. Three trials of copper only experiments with late 3rd and early 4th instar shown in Table 3 were completed. Two trials of copper and silver experiments were performed. At the end of the experiments, live mosquitoes (eggs, larvae, pupae, adults) were frozen overnight before disposal.

Results

MadiDop+ and Copper Mesh Results

Samples collected from the 10L buckets were analyzed for copper and silver concentrations. Both the amount and type of copper mesh were compared in terms of the levels of copper release. Over 24 hour intervals, 10g fine copper mesh released the highest levels of copper, with an average release of 267.5ppb Cu across 10 days, compared to both 5g fine copper mesh and 10g coarse copper mesh (Figure 1). The copper release concentrations from the mesh slightly decreased when a MadiDrop+ was added to the configurations, but the 10g fine copper mesh still released the most amount of copper when in a bucket with a MadiDrop+, with an average release of 250.9ppb Cu across 4 days (Figure 2). Silver concentrations were generally the highest over time when a MadiDrop+ was with 10 grams of fine copper mesh, with an average release of 72.1ppb Ag across 4 days, compared to the MadiDrop+ alone and the MadiDrop+ with other copper mesh configurations (Figure 3).

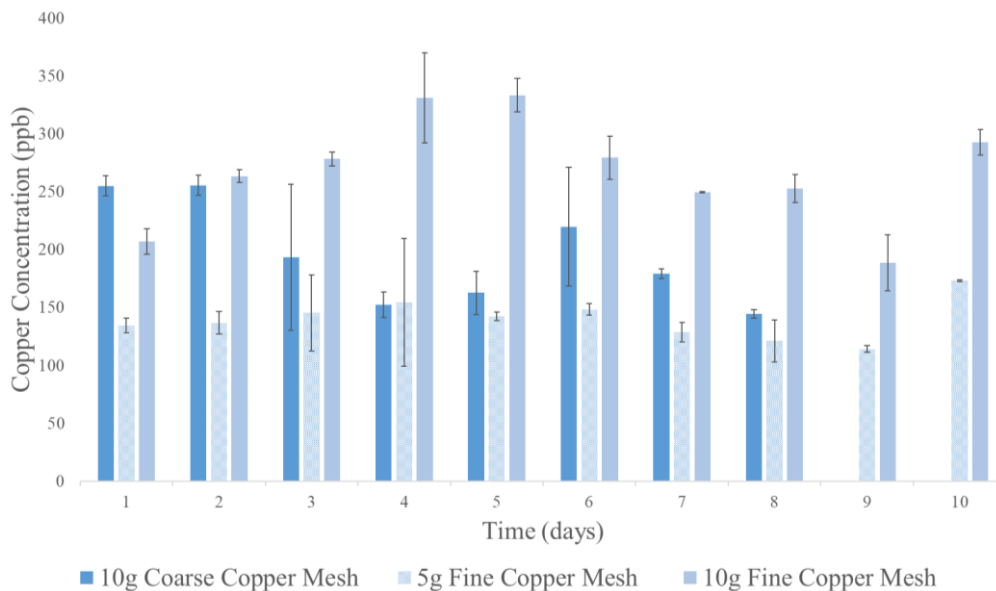


Figure 1: Copper release concentrations from coarse copper mesh and fine copper mesh over time. Two buckets of each configuration (10g coarse copper mesh, 5g fine copper mesh, and 10g coarse copper mesh) were sampled and analyzed to determine the copper release levels over 24 hour time periods. The average of the results from each configuration are shown in this figure.

The 10g fine copper mesh released the greatest amount of copper over time, with an average release of 267.5ppb Cu across 10 days.

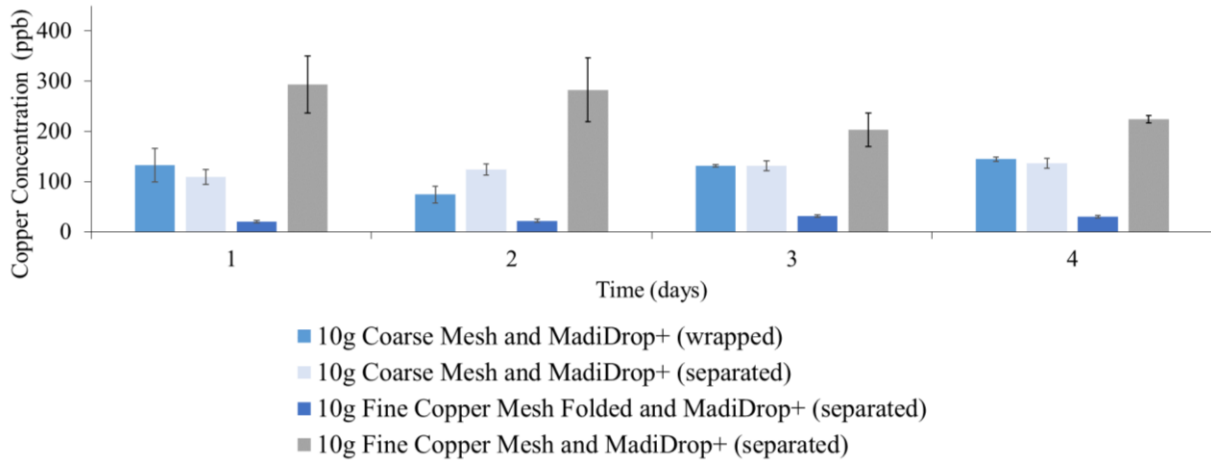


Figure 2: Copper release concentrations from coarse copper mesh with a MadiDrop+ and fine copper mesh with a MadiDrop+ over time. Two buckets of 10g coarse copper mesh wrapped around a MadiDrop+ tablet, 10g coarse mesh alongside a MadiDrop+ tablet, and of 10g fine copper mesh folded alongside a MadiDrop+ tablet, and three buckets of 10g fine copper mesh alongside a MadiDrop+ tablet were sampled and analyzed to determine the copper release levels over 24 hour time periods. The average of the results from each configuration are shown in this figure. The 10g fine copper mesh and MadiDrop+ released the greatest amount of copper over time, with an average concentration of 250.9ppb Cu across 4 days.

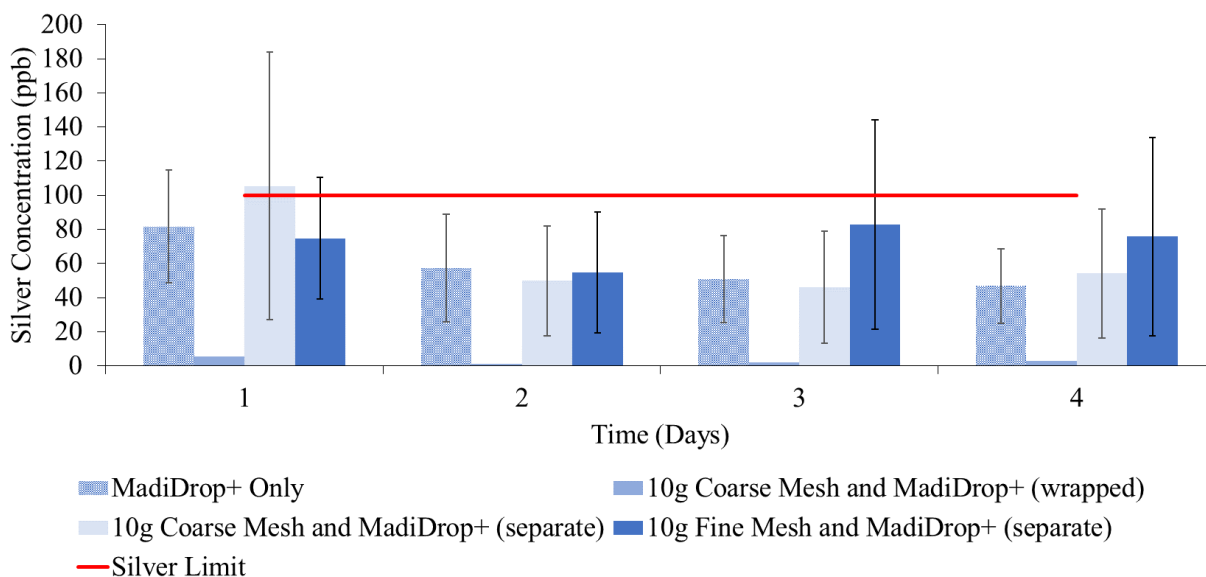


Figure 3: Silver release concentrations from a MadiDrop+, coarse copper mesh with a MadiDrop+, and fine copper mesh with a MadiDrop+ over time. Two buckets of 10g coarse copper mesh wrapped around a MadiDrop+ tablet and of 10g coarse mesh alongside a MadiDrop+ tablet, and three buckets of just a MadiDrop+ and of 10g fine copper mesh alongside a MadiDrop+ tablet were sampled and analyzed to determine the silver release levels over 24 hour time periods. The average of the results from each configuration are shown in this figure. The 10g fine copper mesh and MadiDrop+ released the greatest amount of silver over time, with an average concentration of 72.1ppb Ag across 4 days.

Mosquito Larvae Experimentation Results

Data collected from the mosquito larvae experiments were analyzed to understand the effective levels of copper and silver for mosquito larvae die-off. Over 24 hour intervals, beakers initially containing 25 mosquito larvae and varying concentrations of copper and/or silver were measured to determine the amount of larvae that have died and survived. When using different levels of copper on third instar larvae, 1200ppb Cu was most effective in killing mosquito larvae over time, with between about 40-91% die-off after 14-16 days, compared to 300ppb Cu and 600ppb Cu (Figure 1; Figure 2; Figure 3). When using 600ppb Cu and 40ppb Ag for comparison of these chemicals in terms of effectiveness in second instar larval die-off, 40ppb Ag alone and

600ppb Cu + 40ppb Ag were equally effective in killing mosquito larvae. Both configurations had 100% die off after 16-18 days (Figure 7; Figure 8).

Set 1 - Copper Experiments

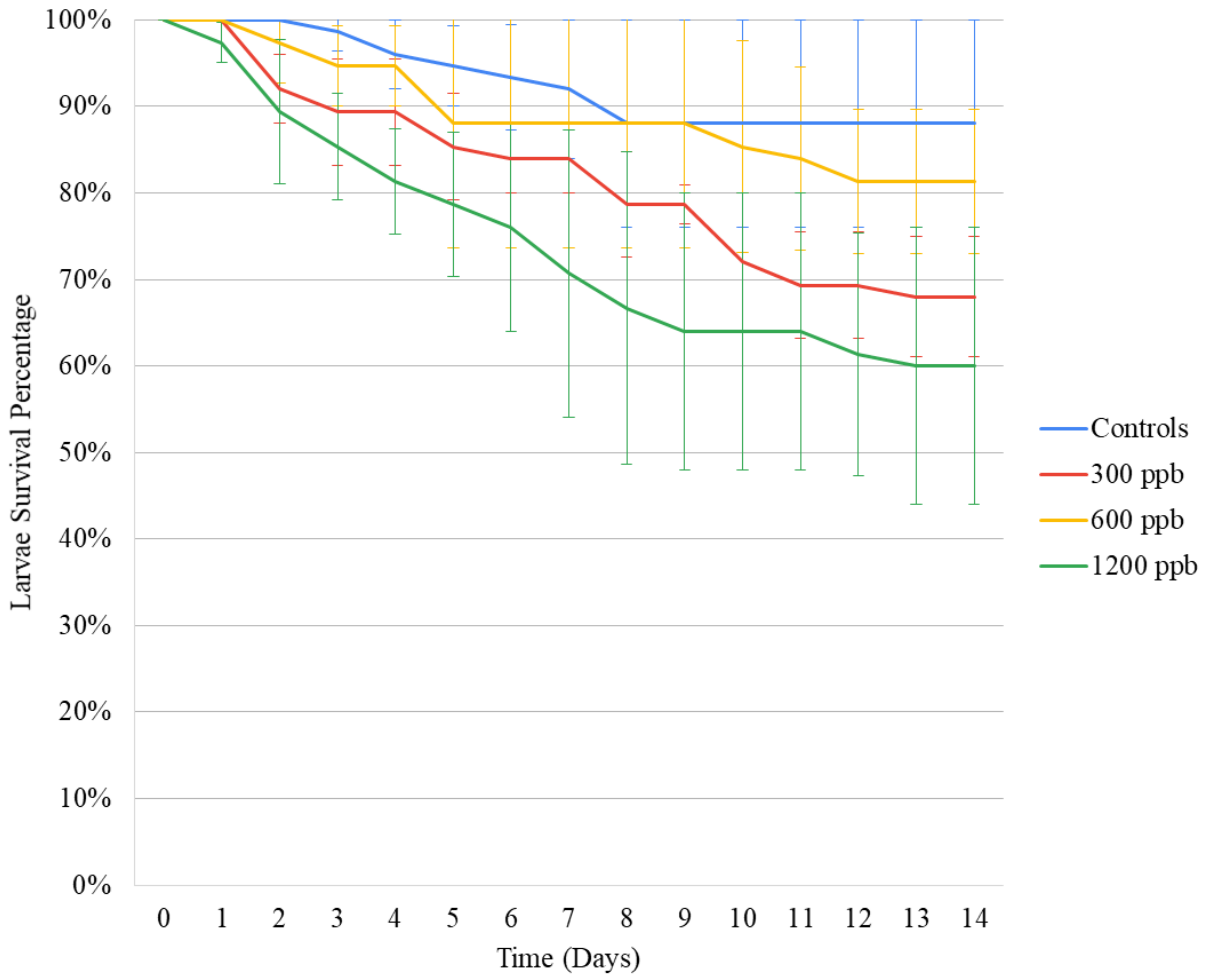


Figure 4: Trial 1 - Third instar mosquito larvae survival over time. Three beakers of each copper concentration (300ppb, 600ppb, and 1200ppb), along with three beakers without copper, were constructed. 25 mosquito larvae were placed in each beaker, and their survival rates were measured over time. Mosquito larvae in beakers with 1200ppb saw the lowest average survival rate at 60%, thus the highest die-off rate, at 14 days.

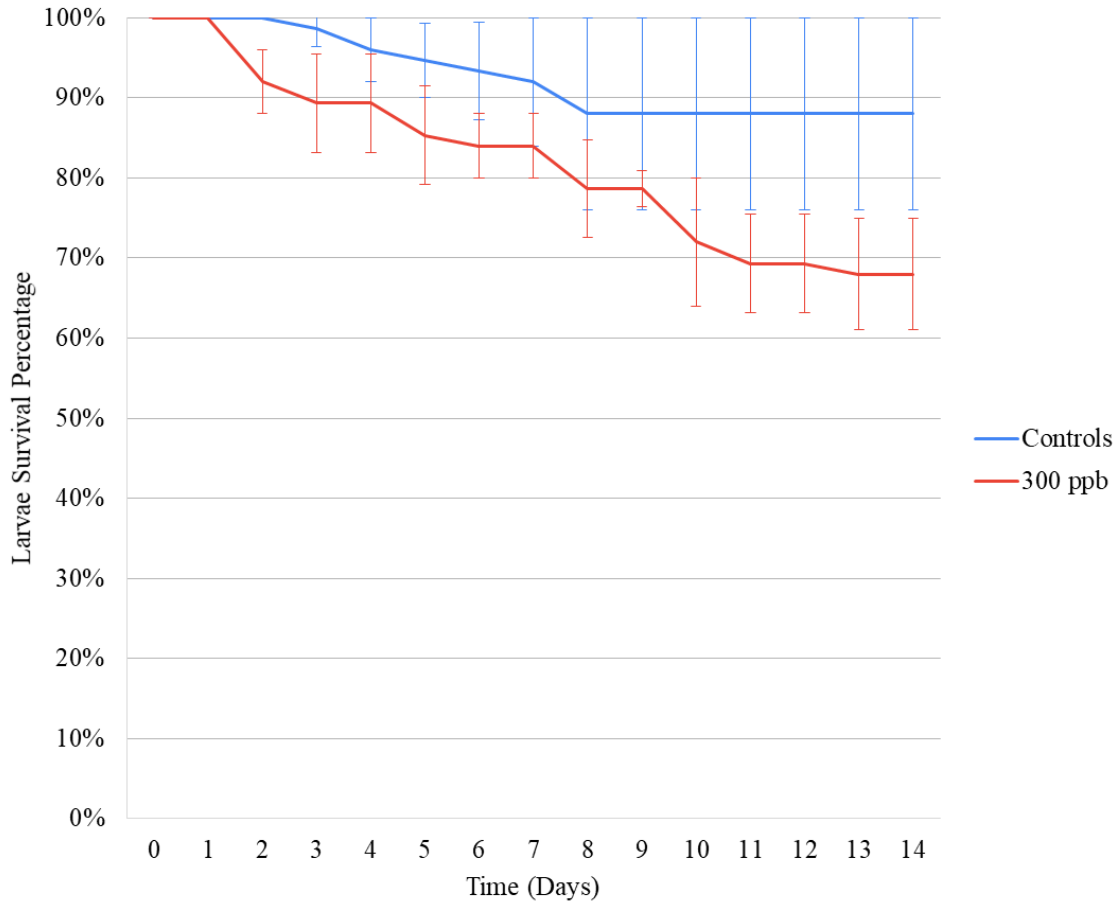


Figure 4.1: Trial 1 graph comparing 300ppb Cu to the control group. Three beakers of both 300ppb Cu and no copper were used with 25 third instar mosquito larvae in each beaker. The survival rates of the larvae were measured over time. The mosquito larvae in control beakers saw an average of 88% survival rate, while the mosquito larvae in the 300ppb Cu beakers saw a 20 percentage point decrease, with an average of 68% survival rate, at 14 days.

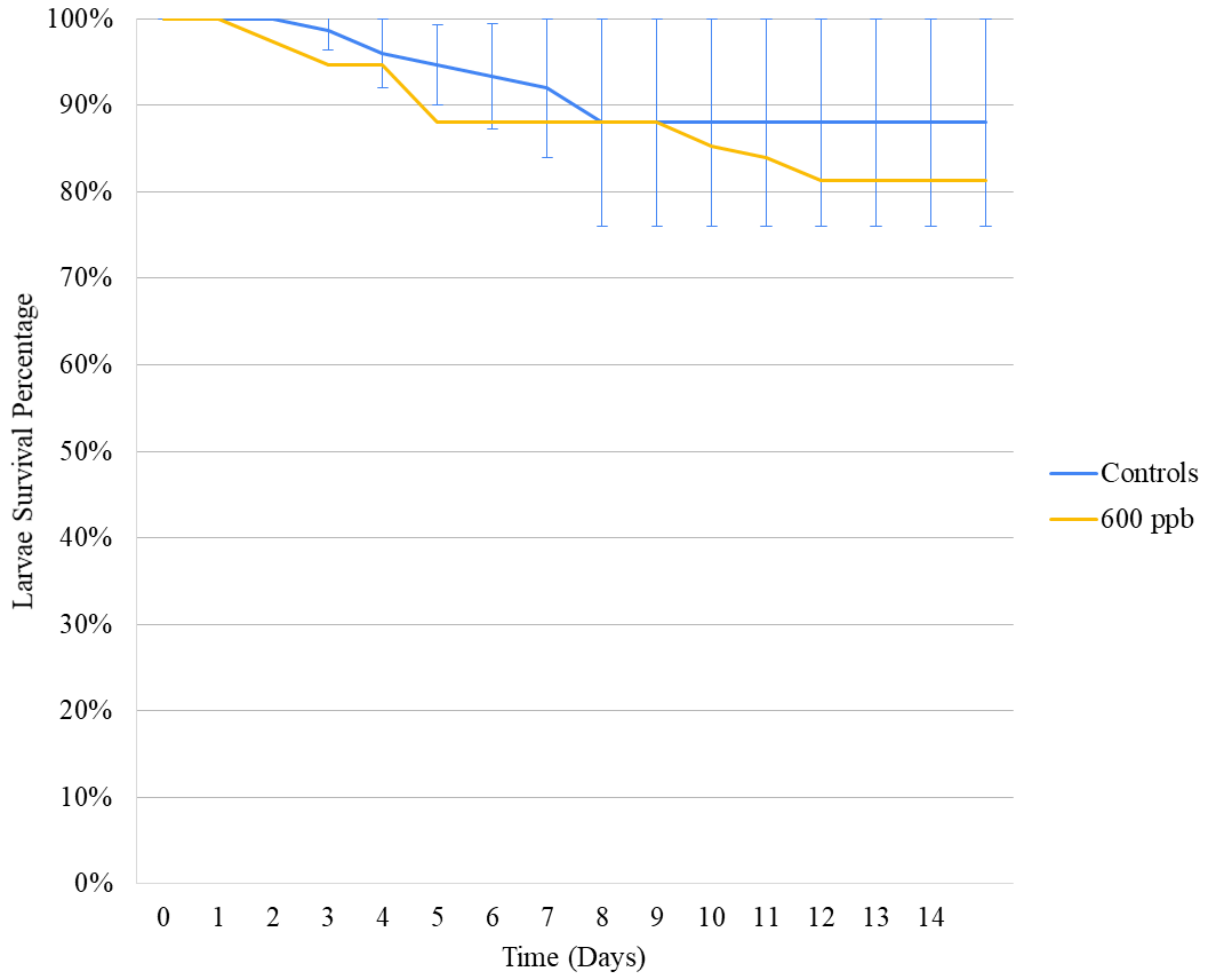


Figure 4.2: Trial 1 graph comparing 600ppb Cu to the control group. Three beakers of both 600ppb Cu and no copper were used with 25 third instar mosquito larvae in each beaker. The survival rates of the larvae were measured over time. The mosquito larvae in control beakers saw an average of 88% survival rate, while the mosquito larvae in the 600ppb Cu beakers saw a 6.67 percentage point decrease, with an average of 81.33% survival rate, at 14 days.

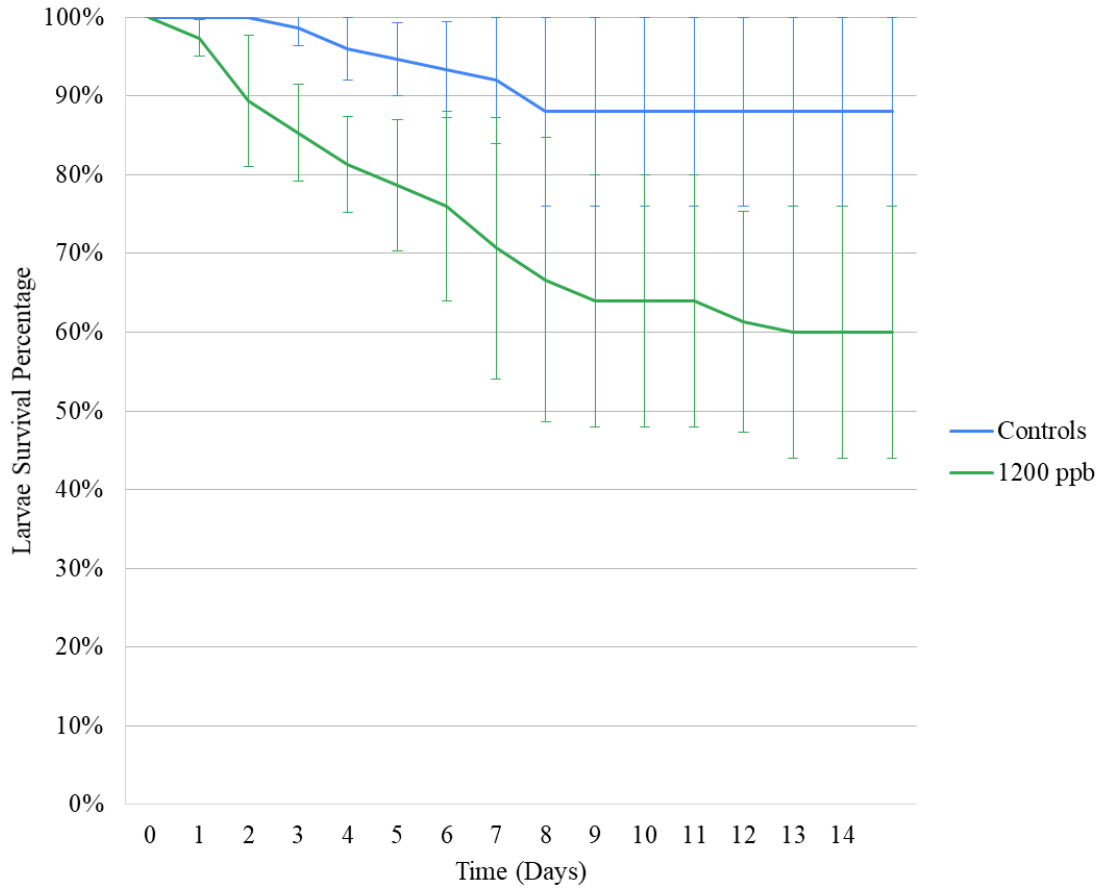


Figure 4.3: Trial 1 graph comparing 1200ppb Cu to the control group. Three beakers of both 1200ppb Cu and no copper were used with 25 third instar mosquito larvae in each beaker. The survival rates of the larvae were measured over time. The mosquito larvae in control beakers saw an average of 88% survival rate, while the mosquito larvae in the 1200ppb Cu beakers saw a 28 percentage point decrease, with an average of 60% survival rate, at 14 days.

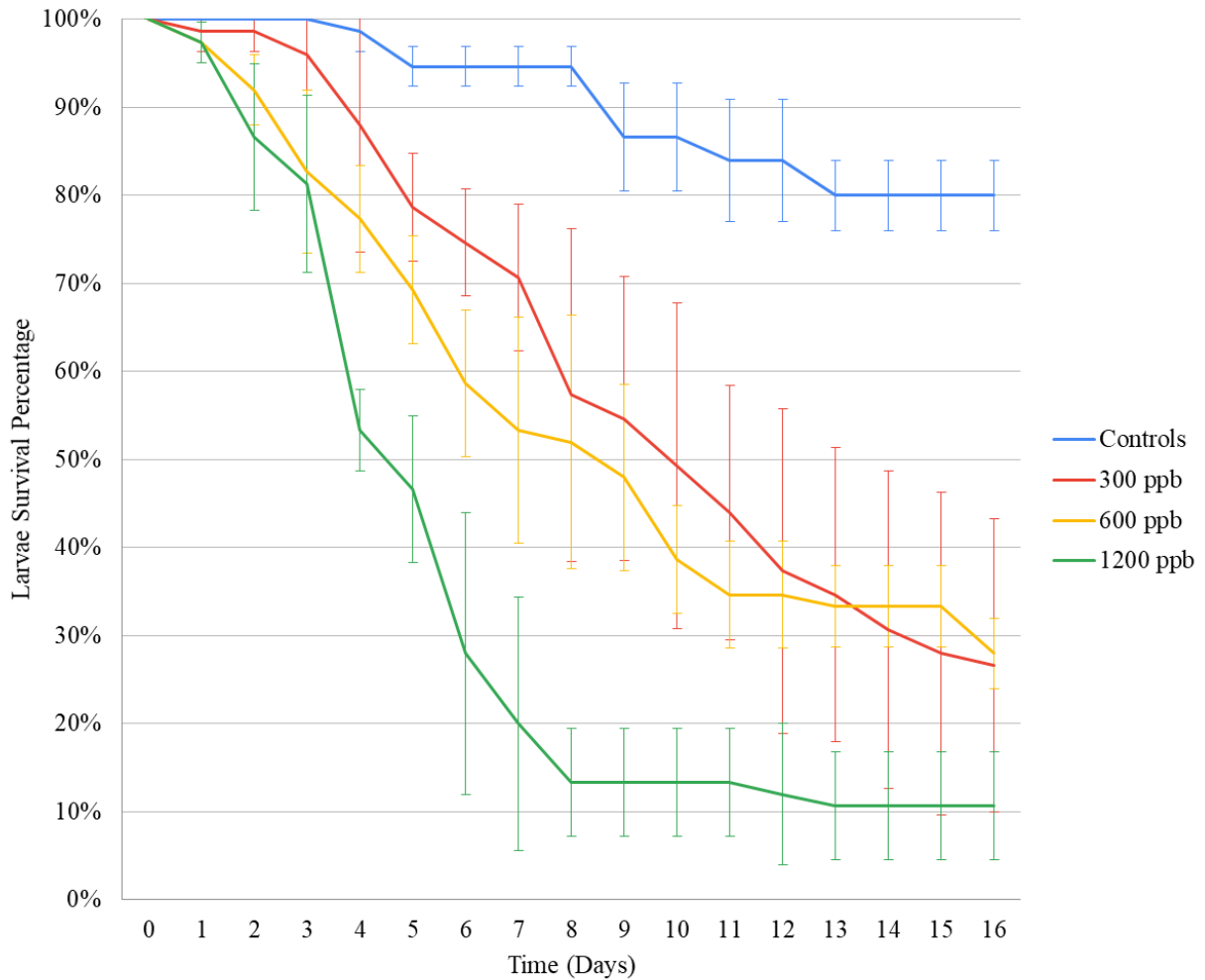


Figure 5: Trial 2 - Third instar mosquito larvae survival over time. Three beakers of each copper concentration (300ppb, 600ppb, and 1200ppb), along with three beakers without copper, were constructed. 25 mosquito larvae were placed in each beaker, and their survival rates were measured over time. Mosquito larvae in beakers with 1200ppb saw the lowest average survival rate at 9.33%, thus the highest die-off rate, at 16 days.

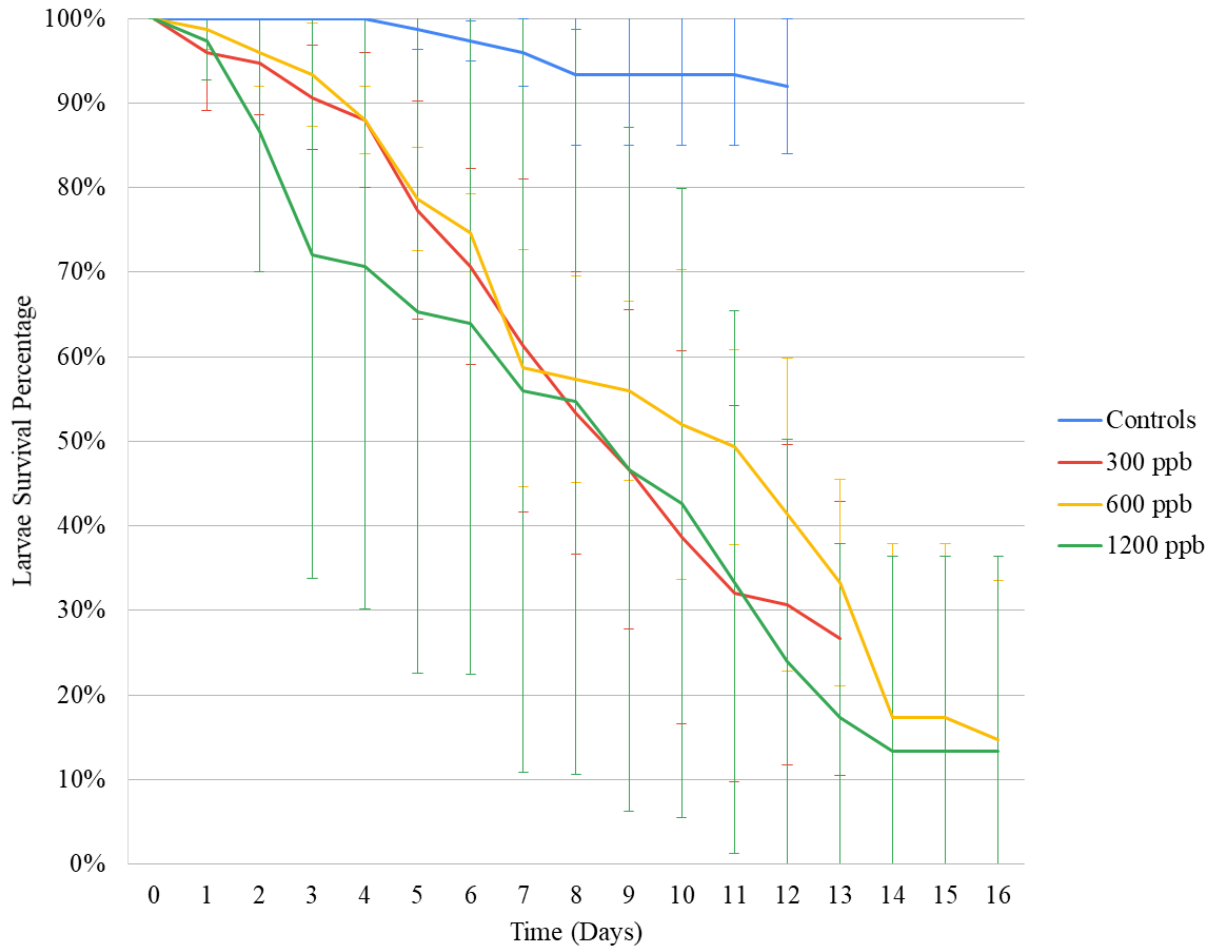


Figure 6: Trial 3 - Third instar mosquito larvae survival over time. Three beakers of each copper concentration (300ppb, 600ppb, and 1200ppb), along with three beakers without copper, were constructed. 25 mosquito larvae were placed in each beaker, and their survival rates were measured over time. Mosquito larvae in beakers with 1200ppb saw the lowest average survival rate at 13.33%, thus the highest die-off rate, at 16 days.

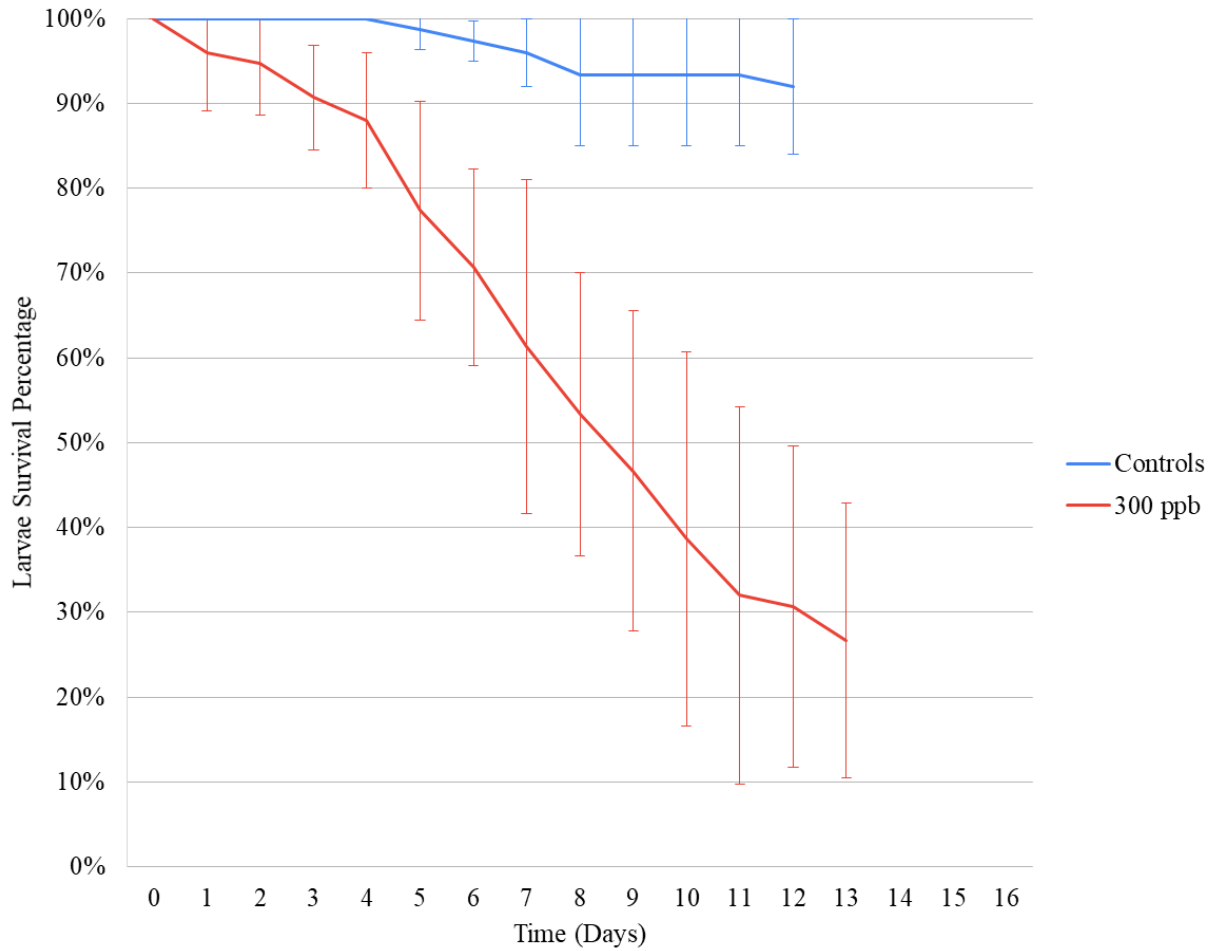


Figure 6.1: Trial 3 graph comparing 300ppb Cu to the control group. Three beakers of both 300ppb Cu and no copper were used with 25 third instar mosquito larvae in each beaker. The survival rates of the larvae were measured over time. The mosquito larvae in control beakers saw an average of 92% survival rate, while the mosquito larvae in the 300ppb Cu beakers saw a 65.33 percentage point decrease, with an average of 26.67% survival rate, at 13 days.

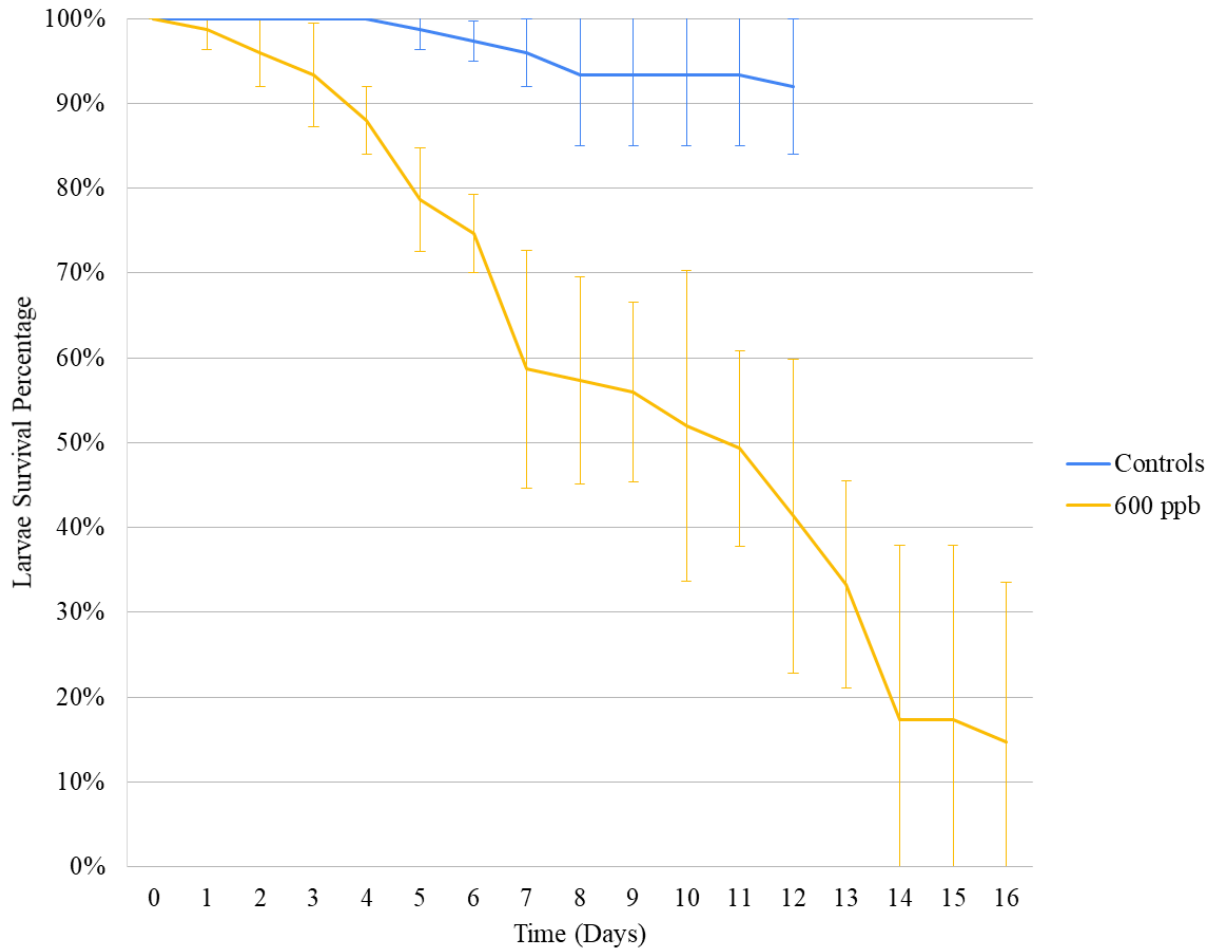


Figure 6.2: Trial 3 graph comparing 600ppb Cu to the control group. Three beakers of both 600ppb Cu and no copper were used with 25 third instar mosquito larvae in each beaker. The survival rates of the larvae were measured over time. The mosquito larvae in control beakers saw an average of 92% survival rate, while the mosquito larvae in the 600ppb Cu beakers saw a 77.33 percentage point decrease, with an average of 14.67% survival rate, at 16 days.

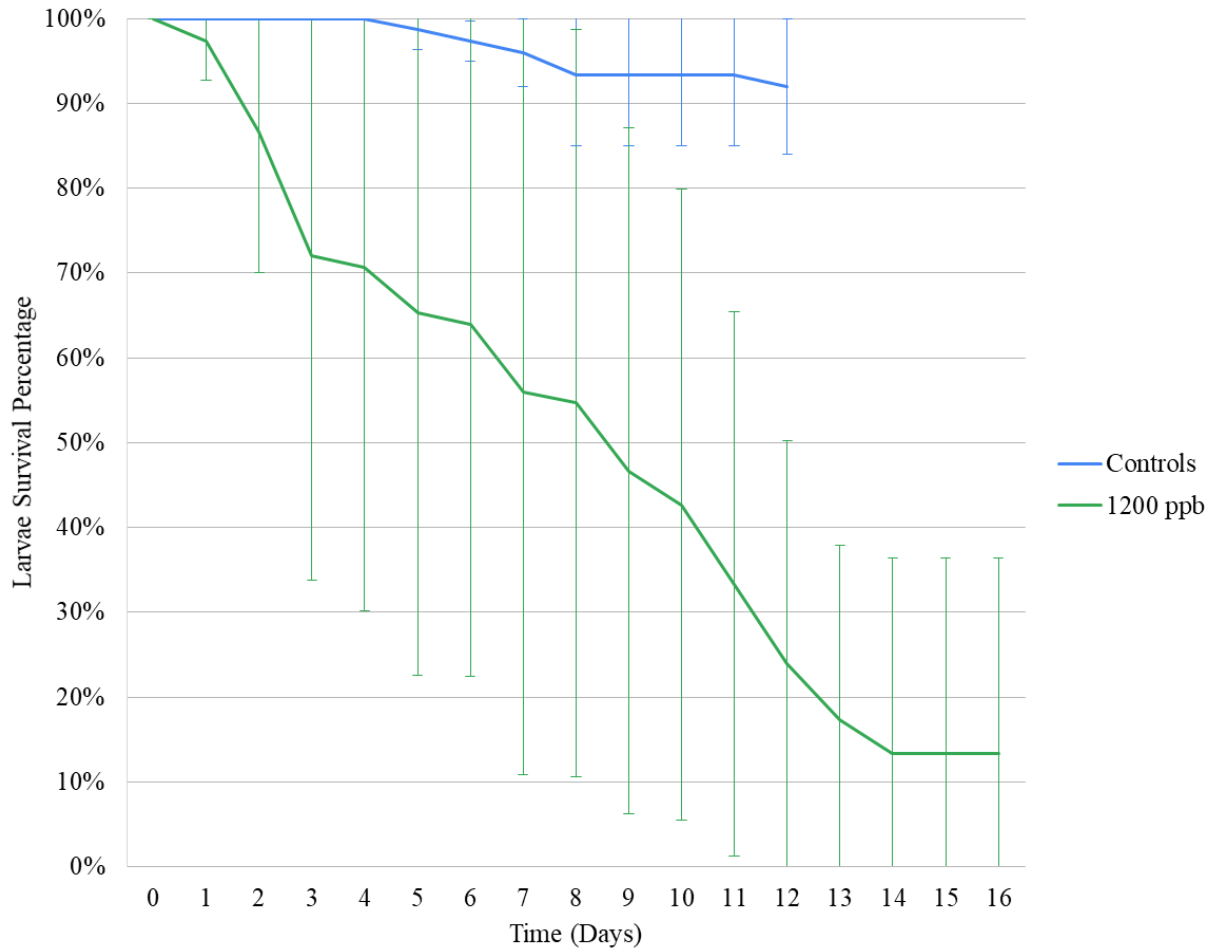


Figure 6.3: Trial 3 graph comparing 1200ppb Cu to the control group. Three beakers of both 1200ppb Cu and no copper were used with 25 third instar mosquito larvae in each beaker. The survival rates of the larvae were measured over time. The mosquito larvae in control beakers saw an average of 92% survival rate, while the mosquito larvae in the 1200ppb Cu beakers saw a 78.67 percentage point decrease, with an average of 13.33% survival rate, at 16 days.

Set 2 - Copper and Silver Experiments

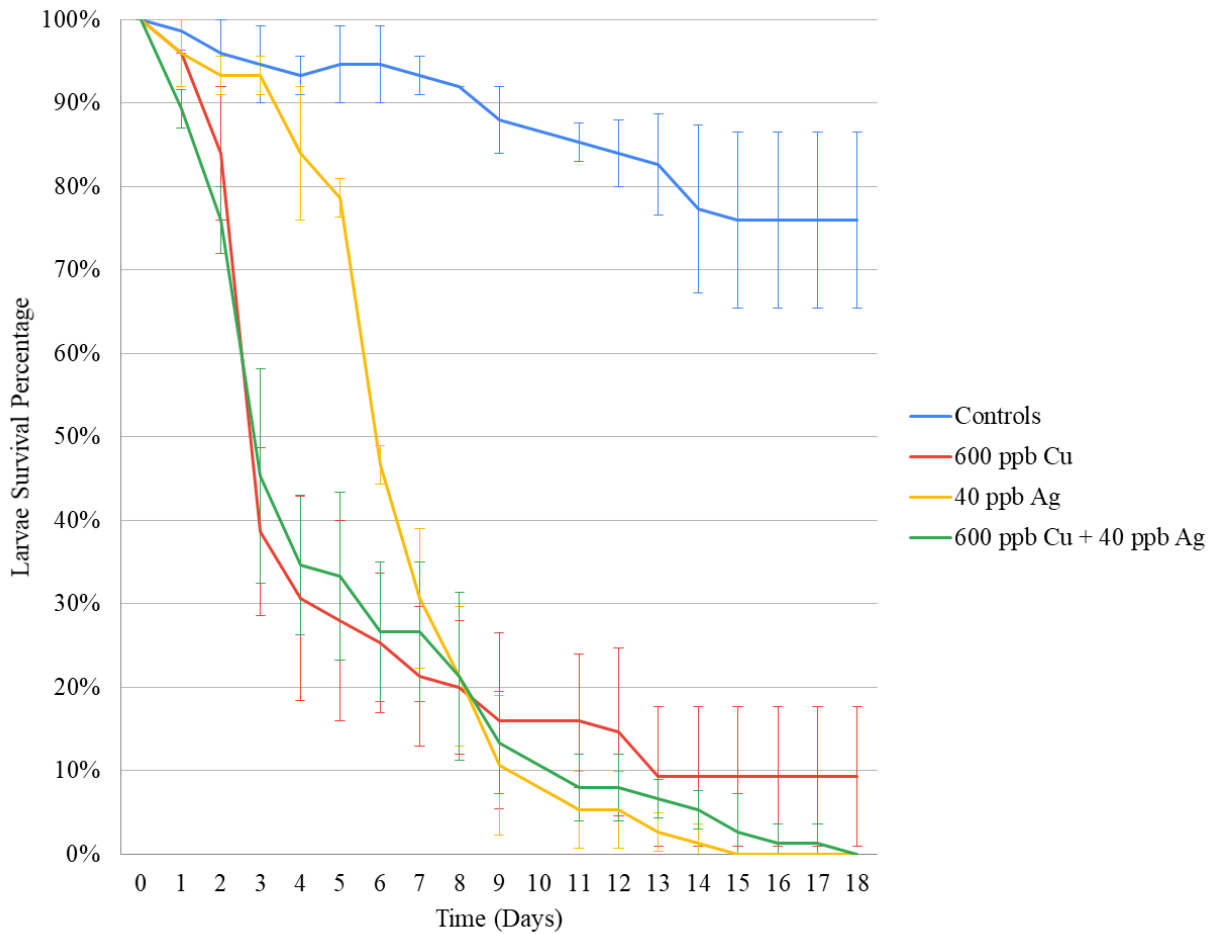


Figure 7: Trial 1 - Second instar mosquito larvae survival over time. Three beakers of each copper and/or silver concentrations (600ppb Cu, 40ppb Ag, and 600ppb Cu + 40ppb Ag), along with three beakers without copper or silver, were constructed. 25 mosquito larvae were placed in each beaker, and their survival rates were measured over time. Mosquito larvae in beakers with 40ppb Ag and in beakers with 600ppb Cu + 40 ppb Ag saw the lowest average survival rate at 0%, thus the highest die-off rate, at 18 days.

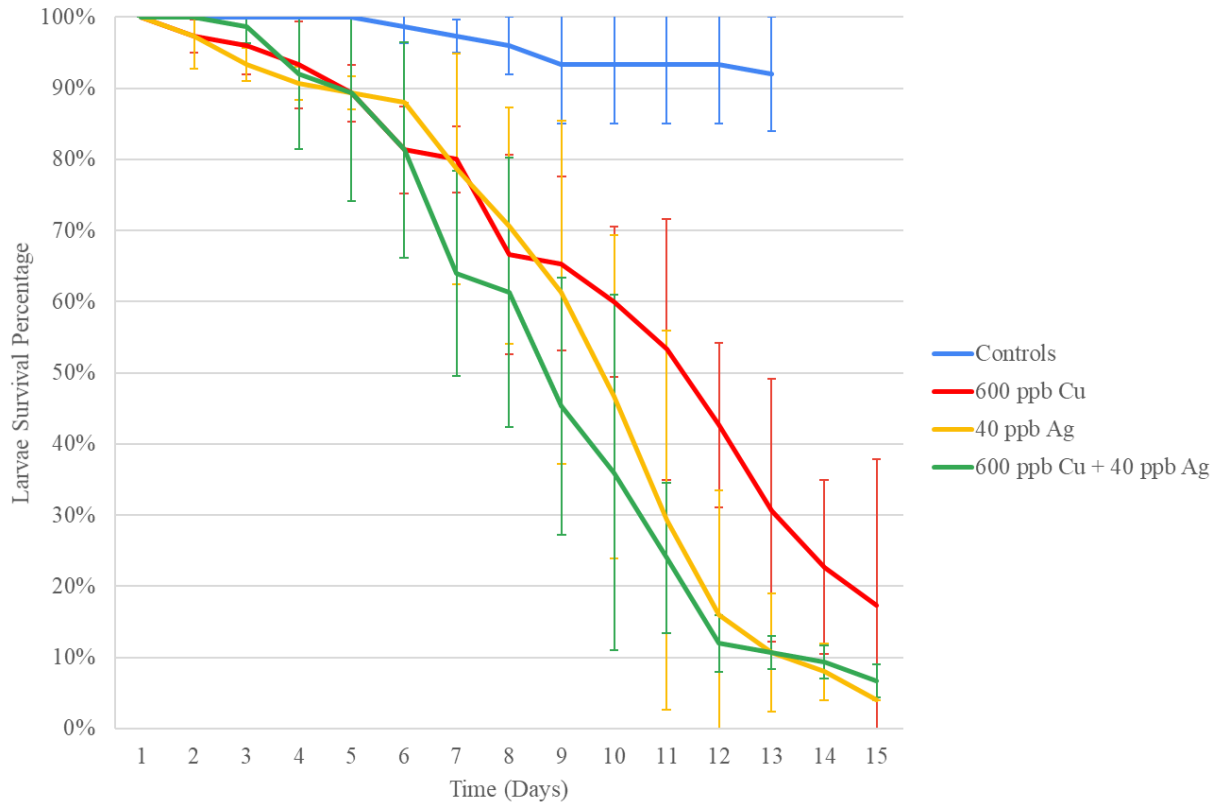


Figure 8: Trial 2 - Second instar mosquito larvae survival over time. Three beakers of each copper and/or silver concentrations (600ppb Cu, 40ppb Ag, and 600ppb Cu + 40ppb Ag), along with three beakers without copper or silver, were constructed. 25 mosquito larvae were placed in each beaker, and their survival rates were measured over time. Mosquito larvae in beakers with 40ppb Ag saw the lowest average survival rate at 5%, thus the highest die-off rate, at 15 days.

Discussion

MadiDrop+

Our results show that the best configuration of copper and the *MadiDrop+* is the 10g fine copper mesh and *MadiDrop+* separated. The target copper release for these configurations was around 600ppb Cu. The fine copper mesh and *MadiDrop+* didn't release enough copper to meet said target concentrations, but it did reach concentrations in the high 200ppb, reaching nearly 300ppb Cu levels (Figure 2). This shows that this configuration has the potential to provide a

higher and consistent release of copper if it were to be altered by adding more grams of copper sheet.

Not only does this configuration show higher and more steady levels of copper release, but it also shows steady release of silver. Having a 10g copper fine copper mesh in the same bucket as the MadiDrop+ but not touching the tablet has consistent silver release, averaging in 72.1ppb (Figure 3). This is a higher silver release than the MadiDrop+ alone, which averages 59ppb Ag release (Figure 3). This is important because providing higher concentrations of copper and silver could provide this synergistic effect for pathogenic disinfection. It is also important to note that these concentrations are below the contaminant limit set by the Environmental Protection Agency.^{10, 11} Therefore, this configuration has the potential to synergistically disinfect water at levels that are safe for human consumption. Copper is also over 10 times less expensive than silver.¹² Thus, it is a feasible and favorable addition to the MadiDrop+ with the goal of water disinfection.

Mosquitoes

It is important to recognize that our experiments were done on different batches of larvae for each experiment. New eggs had to be hatched for each set, and therefore variability of results between each batch of larvae resulted, as we are working with live animals. This is, however, a more realistic simulation to a real-life scenario, since larvae hatching in bodies of water are going to have variability in size, strength, and fitness.

Our first set of experiments on mosquitos was three trials of testing the effects of 300ppb, 600ppb, and 1200ppb copper on mosquito larvae survival. The results of trials 2 and 3 show that a presence of copper solution in the water did have a statistically significant effect on lowering the mosquito larvae survival, seen in Figures 5, 6, and 6.1-6.3. The second trial results show that the larvae exposed to 1200ppb Cu had a lower survival percentage than both the 300ppb Cu and 600ppb Cu as well. These results were not confirmed in the first trial however, which showed no statistical significance between the controls and the different copper concentrations in the water.

Our second set of experiments on mosquitos included two trials testing the effects of 600ppb Cu, 40ppb Ag, and the combination of 600ppb Cu + 40ppb Ag on mosquito larvae

survival. The results of both trials showed statistical significance between any form of exposure when compared to the controls. The first trial starts with a sharp decline in survival for the copper and copper with silver beakers, while the only silver beaker sharply declines similarly but a few days later (Figure 7). All three beakers eventually have under 10% larval survival after 13 days. For the second trial, all three variables have similar survival declines to one another, and all end up with below 20% larval survival by day 15 (Figure 8).

Literature

Previous studies have concluded that copper and silver have synergistic effects in treating microbial infections.⁷ Additionally, research has suggested that copper and silver can act as long-lasting, effective residual disinfection.⁸ This is consistent with the results of the MadiDrop+, which releases silver microparticles that provides residual disinfection. Adding copper in combination with the MadiDrop+ has the potential to further the effectiveness of residual disinfection, thereby motivating this research to determine a successful configuration of copper mesh and a MadiDrop+ tablet. There is a lack of comprehensive research and literature with respect to conclusive effects of copper and silver on mosquito larvae survival and kill-off. However, previous laboratory testing has shown a potent larvicidal effect with less than 1ppm of copper on first instar mosquito larvae.¹³ These results are promising and supportive of our research, but our group aimed to study impacts on varying larvae instar levels. Hence, our group aimed to analyze the separate and combined effects of copper and silver on second and third instar mosquito larvae.

Limitations and Future Work

Our project was constrained to about a seven month time period. Therefore, we were limited in the number of trials we could undertake for each experiment, as well as the duration for each experiment. Our group recommends continuing to collect and analyze samples from the copper mesh and MadiDrop+ tablet experiments. Currently, the MadiDrop+ is effective for a year, so it is crucial for the addition of copper mesh, in any configuration, to successfully release adequate copper levels for the same duration. Therefore, the analysis of silver and copper

concentrations from the 10g fine copper mesh and MadiDrop+ tablet over a year should be completed before concluding that this configuration is successful over time. Additionally, while copper and silver did not appear to have synergistic effects related to mosquito larval kill-off, there is potential for the copper and silver to effectively kill pathogens, such as bacteria. Thus, future work should employ the 10g fine copper mesh and MadiDrop+ configuration in bacteria-contaminated water to analyze the effects of copper and silver on bacterial disinfection for drinking water.

Conclusion

These findings are important for advancing water disinfection and combating the global water crisis. With millions of people around the world not having access to clean, consistent drinking water, advanced technologies that are accessible are crucial. While silver and copper together did not synergistically impact larvicidal rates over time, both of these chemicals were sufficient alone in killing mosquito larvae. These results can support further research into methods of implementation of using copper or silver for disinfection. Additionally, as previous research has suggested synergistic effects from the combination of copper and silver, it was important for us to research methods of implementing copper with the silver embedded MadiDrop+.⁷ Out of the configurations we studied, we found that 10g fine copper mesh alongside a MadiDrop+ was most effective in releasing consistent levels of both copper and silver. These results may help to prompt further research into testing varying gram measurements of copper mesh alongside the MadiDrop+, sampling and measuring copper and silver levels over a longer period of time than our research group was restricted to, and ways for implementing the best configurations for use. Overall, this research has helped to advance our understanding of mitigating the problem of contaminated drinking water.

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