The Effects of Cobalt Sulfate on *Tetrahymena Thermophila* Population Growth Rates

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Abstract

Tetrahymena thermophila is a unicellular eukaryote found in freshwater habitats. Substances released via anthropogenic activity can have detrimental effects on aquatic species such as *T. thermophila*. Specifically, in British Columbia, there are several copper mines that release cobalt as a major by-product. Cobalt sulfate, a heavy metal, enters the water and binds to sediment, resulting in harmful effects on aquatic ecosystems. To study these effects on aquatic organisms, *T. thermophila* was exposed to three different concentrations of cobalt sulfate (0 mg/L, 9 mg/L, and 11 mg/L). Cell counts were taken after exposure to cobalt sulfate for 1.5, 3.5, 5.5, 7.5, 24, 26.5, and 28.5 hours and were used to determine cell growth rates (cells/mL/hour) over 28.5 hours. A One-Way ANOVA test was performed, leading us to reject the null hypothesis that none of the control nor treatment groups are different from each other. Using the Tukey-Kramer test, it was found that the 11 mg/L treatment was significantly different from the 0 mg/L treatment. Instead of having a negative effect on cell growth rates, the data support the idea that increasing concentrations might have been beneficial to *T. thermophila* cell growth. However, there are many limitations to our study, thus, further research needs to be conducted to solidify whether cobalt sulfate is beneficial or detrimental towards *T. thermophila*.

Introduction

Cobalt sulfate is a heavy metal that is frequently used in electrochemical industries (Health Canada, 2017). Various anthropogenic activities result in cobalt sulfate release into the environment including the burning of fossil fuels, wastewater biosolids, and phosphate fertilizers (Health Canada, 2017). However, its main source is industrial processes which include base metal production and alloy/superalloy manufacturing (Health Canada, 2017). Mining has caused a significant increase in the heavy metal content in the atmosphere, as well as in many aquatic and terrestrial ecosystems (Peñuelas and Filella, 2002). There are several copper mines located in British Columbia and cobalt is a typical by-product of copper mining (Health Canada, 2017). Once the sources of cobalt sulfate are released into the environment, they can get into aquatic systems (Health Canada, 2017). Cobalt sulfate is known to have a high solubility in water, thus making it easier for these substances to dissolve once in contact with water (Health Canada,

2017). Furthermore, cobalt is difficult to remove once water is contaminated because it binds to sediments, rocks and soil (Health Canada, 2017). Certain levels of cobalt can be toxic and harmful to aquatic organisms, such as *Tetrahymena* (Health Canada, 2017).

Tetrahymena thermophila is a unicellular eukaryote that is widely distributed in temperate freshwater environments (Collins & Gorovsky, 2005, Lv et al., 2021). Zhang et al. (2013) investigated the effects of Cadmium and Chromium on *Tetrahymena* growth rates, it was found that the growth rate in the presence of these heavy metals was lower than in the control group and that as concentrations of these metals increased the growth rate decreased. Although studies have determined the effects of various heavy metals on the growth rate of *T. thermophila*, little is known about the effects of cobalt sulfate on freshwater organisms such as *T. thermophila.* Our study was conducted to determine the effects of cobalt sulfate on *T. thermophila* growth rates. The model organism of this study, *T. thermophila* has an extremely fast growth rate under simple culture conditions (Orias et al., 1999).

To determine the effects of cobalt sulfate on *T. thermophila*, we grew them under various concentrations. The concentrations used were 9 mg/L and 11 mg/L of cobalt sulfate and the control had 0 mg/L of cobalt sulfate. These concentrations were chosen as they were found to be the limits that *Tetrahymena* can withstand in a previous study (Carter & Cameron 1973). The number of cells in each replicate were counted at 1.5, 3.5, 5.5, 7.5, 24, 26.5, and 28.5 hours. It was hypothesized that *T. thermophila* will show different cell growth rates with varying concentrations of cobalt sulfate. It was predicted that higher concentrations of cobalt sulfate will decrease the cell growth rate of *T. thermophila*.

Methods

Sample Preparation

To study the effects of cobalt sulfate on the growth of *T. thermophila*, 3 replicates were created for each predetermined concentration treatment, totalling 9 replicates. The concentrations the *T. thermophila* were exposed to were 0 mg/L (control), $CoSO_4$ 9 mg/L $CoSO_4$ and 11 mg/L $CoSO_4$. Each replicate was a mixture of 5 mL of *T. thermophila* solution and 5 mL of the respective $CoSO_4$ treatment for a 1:1 ratio (Figure 1). Before any replicates were made, the concentration of stock *T. thermophila* solution provided by the lab had to be counted using a Hemocytometer and it was determined to be $2.8x10^5$ cells/mL. This stock concentration was in the ideal concentration range to start the growth curves, so dilution with *T. thermophila* medium was not necessary. Note that since each replicate is a 1:1 ratio the actual starting concentration of *T. thermophila* is further halved to $1.4x10^5$ cells/L. The stock concentration of cobalt sulfate used for this experiment was 22 mg $CoSO_4$/L. To prepare the control replicates, 5 mL of working *T. thermophila* stock is pipetted into 3 test tubes along with 5 mL of *T. thermophila* medium. To prepare the 9 mg/L cobalt sulfate treatment, 4.09 mL of the stock cobalt sulfate was pipetted into 3 test tubes along with 0.91 mL of *T. thermophila* medium and 5 mL of working *T. thermophila* stock. To prepare the 11 mg/L cobalt sulfate treatment, 5 mL of stock cobalt sulfate was pipetted into 3 test tubes along with 5 mL of working *T. thermophila* stock. Once samples were created they were stored in a 30℃ incubator, the optimal growth temperature for *T. thermophila*, and only taken out for counting.

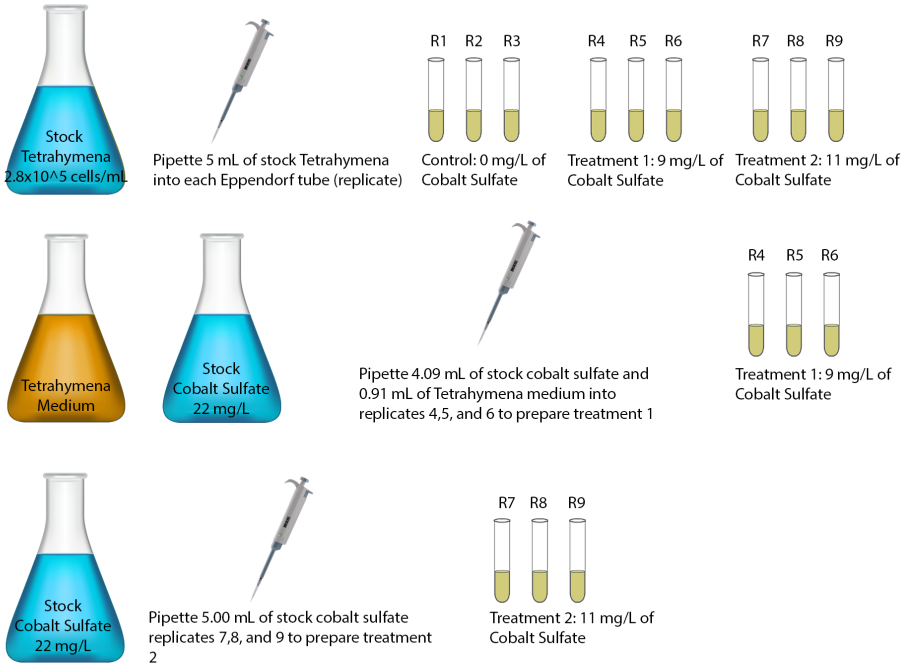

Figure 1. Prepared stock culture of *T. thermophila* was pipetted into each test tube and separated into 3 treatments each with 3 replicates. The amount of medium and cobalt sulfate added to the test tubes differed between the control, treatment 1, and treatment 2.

Treatment Sampling

The test tube tray containing the 9 replicates was removed from the 30℃ incubator during each sampling period. Each sample was mixed gently by swirling the test tube for roughly 10 seconds. Next, the opening of the test tube was sterilized by swiping it under an alcohol lamp a few times. As shown in Figure 2, 10 µL of fixative was then pipetted into the Eppendorf tube followed by 100 µL of the respective sample. The solution was gently mixed by pipetting the mixture up and down to ensure homogeneity. This was done for each sample, and 63 counts were done in total over 28.5 hours. Samples were taken at 1.5, 3.5, 5.5,7.5, 24, 26.5, and 28.5 hours. The test tube tray with the samples was then returned to the 30℃ incubator until the next count, and the Eppendorf tubes containing the fixative and the samples were placed in the fridge.

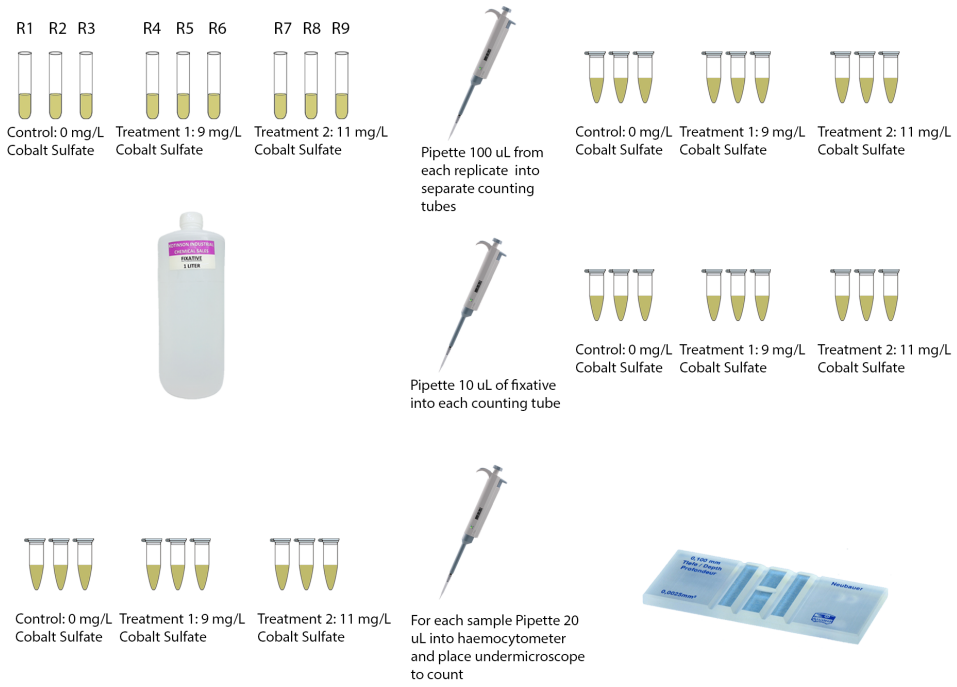

Figure 2. Procedure for sampling each replicate. Each sample was pipetted into separate Eppendorf tubes along with a fixative. The fixed sample from each Eppendorf tube was then pipetted into the Haemocytomer for counting.

Cell Counting

A pipette was used to load 20 µL of a fixed sample from the Eppendorf tube to the hemocytometer (Figure 2). The hemocytometer was then put under the Zeiss microscope and switched to the 10X objective lens (Figure 3). Cells were counted relative to the red squares of the hemocytometer. A tally counter was used to count and counting stopped when the cell count reached 150 cells or all 16 squares were counted. Data was recorded as cells counted/number of squares. This was repeated for each replicate taken at each period.

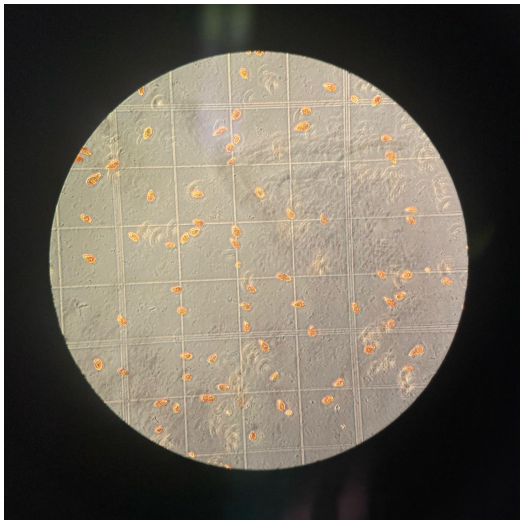

Figure 3. Image of fixed *Tetrahymena thermophila* cells on a hemocytometer taken at 10X on the Zeiss AxioLab microscope.

Data analysis

Once the data was acquired from counting the cells, a linear regression was done for each replicate. The slopes from each replicate were then grouped by treatment level and a one way ANOVA test was done on them to return a p-value. A Tukey-Kramer analysis was then done on the data to determine which groups were significantly different from each other. Lastly, the slopes from the linear regression of the replicates were grouped by treatment level and the means were put on a scatter plot.

Results

The growth rates for the 3 replicates of the control (0 mg/L) were -580.3, -1003.0, and -809.4 (cells/mL/hour). The growth rates for the 3 replicates of the first treatment (9 mg/L) were 544.2, 471.0, and -1007.2 (cells/mL/hour). While the growth rate for the 3 replicates of the second treatment (11 mg/L) was 1457.7, 1768.6, and 817.1 (cells/mL/hour). The mean growth

rates (cells/mL/hour) of 0 mg/L, 9 mg/L and 11 mg/L treatments were -797.6, 2.7, and 1347.8, respectively. In Figure 4, the 11 mg/L has the highest average growth rate, followed by the 9 mg/L, with the control (0 mg/L) having the lowest average cell growth rate.

A one-way ANOVA test was conducted and the p-value was found to be 0.012, which is less than the significance level of 0.05, suggesting this result was statistically significant. A Tukey-Kramer statistical test was also performed. The mean cell growth rate of *T. thermophila* was not significantly different between the control (0 mg/L) and the 9 mg/L treatment (P=0.625), as well as between the 9 mg/L and 11 mg/L treatments (P=0.128). However, the mean cell growth rate of *T. thermophila* was significantly different between the control (0 mg/L) and 11 mg/L treatment (P=0.128).

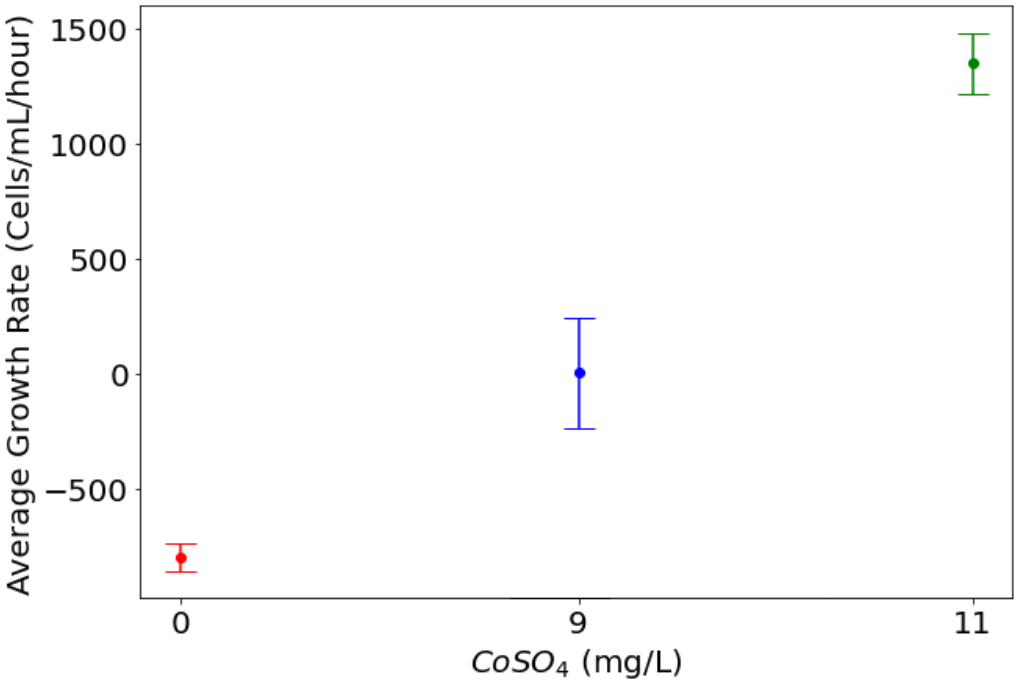

Figure 4. Average growth rate of *Tetrahymena thermophila* (cells/mL) over 28.5 hours in three different treatments of Cobalt Sulfate (0 mg/L, 9 mg/L, and 11 mg/L). Error bars represent 95% Confidence Intervals. (n=3) for each treatment of cobalt sulfate. P-value = 0.012.

Discussion

The growth rates of organisms are an important area to study as it is integral to the stability of their population. The motivation for this study is based on the notion that extracting natural resources can be detrimental to ecosystems. Our goal is to understand whether mining activities in British Columbia affect the surrounding aquatic ecosystems; interpreting the data from our project could indicate whether future regulation changes are required. Cobalt is a common by-product of copper mining and other extraction processes, thus, it is important to experiment with whether leaching of these compounds would affect commonly found aquatic organisms, such as *T. thermophila*. As mentioned previously, the cobalt sulfate becomes near impossible to remove after it becomes attached to the sedimentary layer of water. Past studies have shown that various heavy metals are detrimental to the growth of *T. thermophila*, but they do not prioritize experimentation on growth rates with cobalt sulfate. Based on the limitations provided by Carter et al. (1973), it was decided that this study should examine the effects of cobalt sulfate on *T. thermophila* growth rates at 0 mg/L, 9 mg/L, and 11 mg/L. This study can provide introductory knowledge of whether the popularity of mining in B.C. could impact the ecosystem negatively.

Discussion of Results

Our hypothesis was established with the previous knowledge that other heavy metal experimentations were detrimental to the growth rate of *T. thermophila.* After tabulating the data, quantitative observations portray that increasing the concentration of cobalt sulfate may be beneficial for *T. thermophila* as we see increases in growth rate at different times. Averaging the cell count for each replicate of each respective treatment from the first sampling time (1.5hrs) to the last sampling time (28.5 hrs), establishes that the highest average number of cell counts

occurred in the 11 mg/L treatment while the lowest average number of cell counts occurred in the control. This initial assessment of the cell count averages indicates that increasing the concentrations of cobalt sulfate may increase the growth rate of *T. thermophila.*

Before any statistical tests were done a linear regression was performed to determine the slopes of each replicate. We did a one-way ANOVA test to compute the p-value from the slopes of each replicate. It was found that the p-value was less than 0.05, thus, we reject the null hypothesis; there is at least one group amongst the three with significant differences in growth rate. Supported by the Tukey-Kramer test, it is clear that the 11 mg/L treatment had a significant difference; the test displayed significant differences between the control and treatment 2 (11 mg/L). The fact that this treatment group was significantly different from others and had a higher trending growth rate further implies that increasing the concentration of cobalt sulfate is advantageous. A possible reason for this result could be that cobalt sulfate helps *T. thermophila* with the metabolism of the medium, however, further studies are required as there is minimal evidence for this rationale.

From the ANOVA and Tukey-Kramer analyses, the results do not support the idea that increasing the concentrations of cobalt sulfate have a detrimental effect on the growth of *T. thermophila*. The study by Carter et al. (1973) found that increasing concentrations of cobalt sulfate up to 10 mg/L of cobalt sulfate did impact the growth rate of *T. thermophila* negatively, thus, this finding led us to use a slightly more potent concentration (11 mg/L) to attempt and recreate similar results. Our attempt to derive similar results was not achieved. A possible reason for this is that Carter et al. (1973) used a maximum cell count of 110 cells while our study used much greater populations. As mentioned previously, some cells may have the ability to adapt to the cobalt sulfate conditions, and having more cells increases the chances of having resistant

cells. It could also be that in Carter et al. 's (1973) study, they used an undisclosed medium in their solution, and the cobalt sulfate's interaction with their undisclosed medium could have created hazardous conditions for the growth rate of *T. thermophila* in their study. We only exposed the *T. thermophila* to cobalt sulfate for a maximum of 28.5 hrs before the final sampling, while their study had their final sample after 96 hrs. This sampling difference could have prevented our study from replicating similar results. Overall, our results indicate that cobalt sulfate may have significantly beneficial effects at increasing levels of cobalt sulfate.

Sources of Error and Limitations

There are several potential sources of error for this experiment. Cell counts are one of the potential sources of error. Although there were methods to keep the counts consistent, each student introduced their own counting bias. Additionally, before counting the cells, the samples were mixed by gently pipetting up and down. Even after mixing the sample thoroughly, there were still large clumps of cells which resulted in an inaccurate distribution of cells on the hemocytometer which affected our overall cell count. This study was limited by sample size, there were only 3 replicates each for the 2 treatment groups and control, thus decreasing the statistical power. There was also a 16.5 hour period during our experiment where samples were not taken at all, thus we cannot account for any fluctuations in the growth rate during that period. Lastly, another limitation is the time periods the cell counts were taken, they were not taken consistently, and they were only taken for a total of 28.5 hours. All of these factors could have impacted the results of our experiment.

Impact of the study on BC ecosystems

As stated earlier, mining activity in British Columbia is quite significant, and the leaching of by-products can be harmful to ecosystems nearby. Understanding the implications of

by-products such as cobalt sulfate on aquatic organisms is important as it can be related to similar keystone species. For our study, we limited our concentration to 11 mg/L of cobalt sulfate due to the findings of Carter et al. (1973), but further research with higher concentrations of cobalt sulfate could be used to replicate the experiment and determine the effect. It is important to test greater concentrations of cobalt sulfate as by-products can accumulate to higher levels in nature that surpass 11 mg/L.

Conclusion

The results of our study demonstrated that higher concentrations of cobalt sulfate positively affected *T. thermophila* cell growth rates. Conducting a one-way ANOVA test, the p-values between the treatments were less than 0.05. Therefore, the null hypothesis was rejected, which supports at least one difference in cell counts between the two treatments and the control. The Tukey-Kramer test establishes that the 11 mg/L concentration was the only statistically significant treatment group. Compared to previous studies, we did not achieve similar results and when reviewing the limitations, there were unique variables that contributed to deficits within our study. Unfortunately, due to these deficits, it is hard to conclude whether cobalt sulfate does have an impact on ecosystems in close proximity to mining sites around British Columbia.

Acknowledgement

We would like to first acknowledge the BIOL 342 teaching team, Celeste Leander and Tessa Blanchard who made this work possible through their ongoing guidance and support. We would also like to thank Mindy Chow and Jarnail Chandi for their assistance in the preparation and planning of our project. Additionally, we would like to extend our appreciation to the University of British Columbia for providing the opportunity and space to conduct our study. Finally, we acknowledge the Musqueam nation for allowing us to learn on their ancestral, unceded and traditional territory.

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