The Effect of Copper Sulfate on the Growth of *Chlamydomonas reinhardtii*

Balkaran Dhaliwal, Karnvir Dhillon, Olivia Kochhar, Hannah Salcudean, Adam Soliman

Abstract

Micro-alga such as *Chlamydomonas reinhardtii* are a key food source for salmon. Therefore, this study aims to investigate the effects of water contamination on *C. reinhardtii* as an indication of how salmon are affected by metal pollutants. *C. reinhardtii* cells were grown in three concentrations (0μM, 10μM, 150μM) of copper sulfate. The cells were counted over 5 days and growth rates were determined for each concentration. No significant difference was found between the growth rates of *C. reinhardtii* cells at each concentration. However, it was observed that an increase in copper sulfate concentration caused a decrease in the average growth rate. The observed results are likely due to uncertainties in haemocytometer counts and potential errors in sample preparation. Therefore, further repetitions of this study are needed in order to determine the potential effect of copper sulfate on *Chlamydomonas reinhardtii*.

Introduction

Salmon are a keystone species in British Columbia as they play a significant role in local food chains, ecosystem function, and nutrient cycling. Recently, Rensel et al. (2010) found that algal blooms in the Fraser river caused a decline in salmon survival and migration numbers. Therefore, it is clear that fluctuating algae levels can significantly impact local salmon populations. There are many causes of algal blooms, but water contaminants are a key contributor to fluctuations in algae numbers.

One contaminant of particular interest is copper, due to its presence in local rivers after the Britannia Mine pollution event in BC. Although the mine stopped production in 1974, the effects can still be seen today. In fact, McCandless (2015) states the mine was "Canada's largest point source of metal pollution" until 2005. Since then, little research has been conducted to determine the effect of copper water contamination on salmon food sources – namely micro-alga. As such, this study aims to investigate the effects of copper contamination on the micro-alga *C. reinhardtii*, a key model organism due to its rapid growth and ability to grow in dark environments using acetate (Sasso et al. 2018). Since salmon rely on micro-alga like *C. reinhardtii*, investigating its growth in the presence of copper will allow for a better understanding of how copper contamination affects salmon populations in British Columbia.

Due to its solubility in water, copper sulfate will be used in this experiment to achieve accurate concentration levels. Previous experiments by Jamers et al. (2013) found that *C. reinhardtii* grows optimally at copper sulfate concentrations of 8μM and that lethal amounts exceed 125μM. Using these values as a benchmark, we will investigate three concentrations of copper sulfate: 0μM, 10μM, and 150μM. Additionally, Jiang et al. (2016) found that

copper concentrations affect *C. reinhardtii* in a dose-dependent manner, where higher concentrations cause more toxic effects. These effects included inhibition of cell growth, decreased chlorophyll content, and increased activity of antioxidant enzymes. Therefore, we hypothesize that changing copper sulfate concentrations will affect the growth rate of *C. reinhardtii*. Furthermore, it is predicted that increasing concentrations of copper sulfate will decrease the growth rate of *C. reinhardtii* cells in this study.

Methods

Separate flasks of 10mL of 300µM copper sulfate, 60mL of standard media, and 30mL of *C. reinhardtii* cultures were received from the University of British Columbia biology lab. An initial count of the *C. reinhardtii* solution using a haemocytometer found the cell concentration to be 3.85×10^5 cells/mL. According to Harris et al. (2009), the optimal concentration for *C. reinhardtii* stock solution is 5.0×10^4 cells/mL. In order to achieve this concentration, a solution of 3.9 mL of *C. reinhardtii* stock was combined in an Erlenmeyer flask with 26.1 mL of standard media (Image 1).

The lab bench was sanitized with 70% ethanol, and gloves and lab coats were worn for the entirety of the experiment. At the lab bench, 9 tubes were labelled for the 3 replicates of control (0µM $CuSO_4$), 3 replicates of 10µM $CuSO_4$, and 3 replicates of 150µM $CuSO_4$ (Image 2). The necessary dilutions were calculated using the $C_1V_1 = C_2V_2$ equation. The standard media, *C. reinhardtii* stock, and $CuSO_4$ were distributed to the tubes accordingly (Image 1) and all samples were flamed before and after distribution in order to reduce contamination. The 9 tubes were placed in a 25℃ incubator for 3 days to allow the *C. reinhardtii* cells to begin the growth phase. After the 3 days, 9 Eppendorf tubes were prepared (one for each replication) with 50µL of *C. reinhardtii* and 5µL of IKI fixative. Each Eppendorf tube was vortexed and 10µL of the sample was loaded onto a haemocytometer slide. Using a compound microscope with the 10x lens, each sample was counted from the bottom right corner of the grid, using a tally counter to reduce error. The dilution factor for each count was used to calculate the cell density of the sample. Each sample was counted three times and the average cell density was recorded. The cell counts for the 9 samples were repeated for 5 consecutive days.

Once all cell counts were completed, the data was entered into an Excel spreadsheet where graphs of the 0µM, 10µM, and 150µM $CuSO_4$ were created by plotting *C. reinhardtii* cell concentration against time. Then, growth rates for each replication were found using the slope of each trendline. With these growth rates, a single-factor ANOVA test and a

Kruskal-Wallis test were performed on R. point-plots comparing the growth rates under 0µM, 10µM, and 150µM $CuSO_4$ were also created using R.

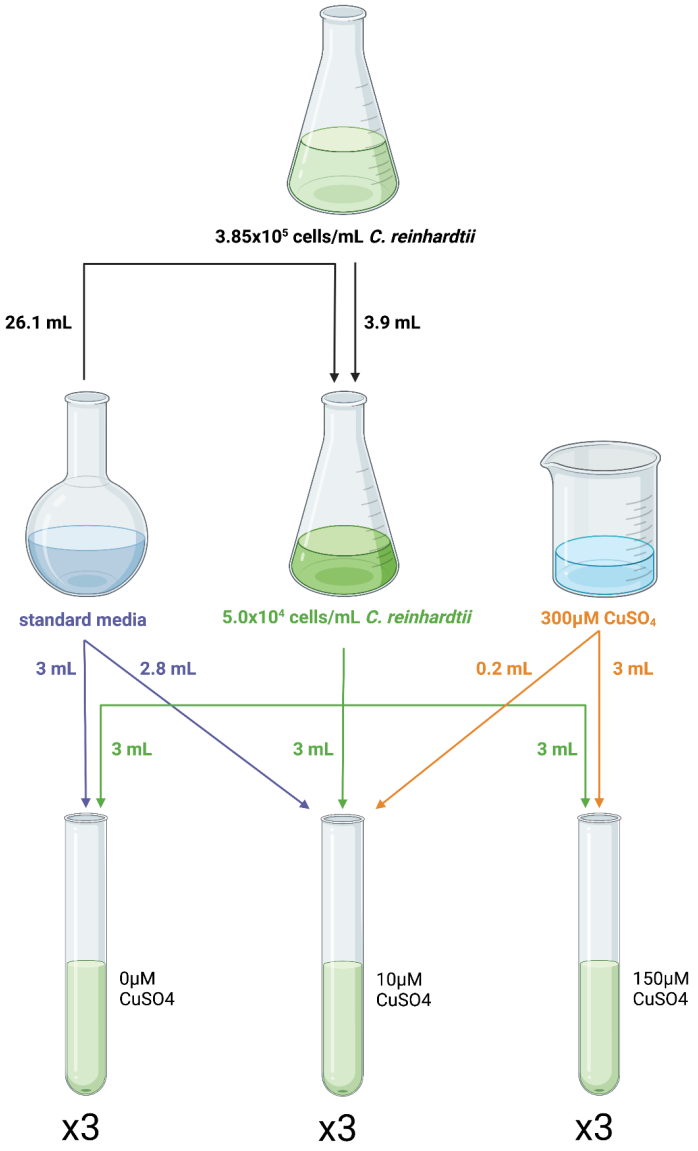

Image 1: Methods of sample preparation for the 9 replications.

Results

In our study, we compared the growth rate of *C. reinhardtii* in three different copper sulfate conditions (0µM, 10µM, and 150µM) using three replicates for each treatment group (n=9). Due to the limited number of replicates, we were unable to approximate a normal distribution in the data, and conducted both a single-factor ANOVA and Kruskal-Wallis test (both with $\alpha = 0.05$). Neither statistical test found a significant difference between the different treatment groups; the single-factor ANOVA and Kruskill-Wallis tests obtained p-values of 0.09055 and 0.06081 respectively. Nevertheless, it was observed that with an

increase in copper sulfate concentration the average growth rate decreased (Figure 1). Notably, one of the replicates at 150μM copper sulfate had a negative growth rate indicating cell death from the first day of cell counting. The magnitude of this negative rate was substantially greater than that of the other two replicates (with positive growth rates) creating a mean negative growth rate.

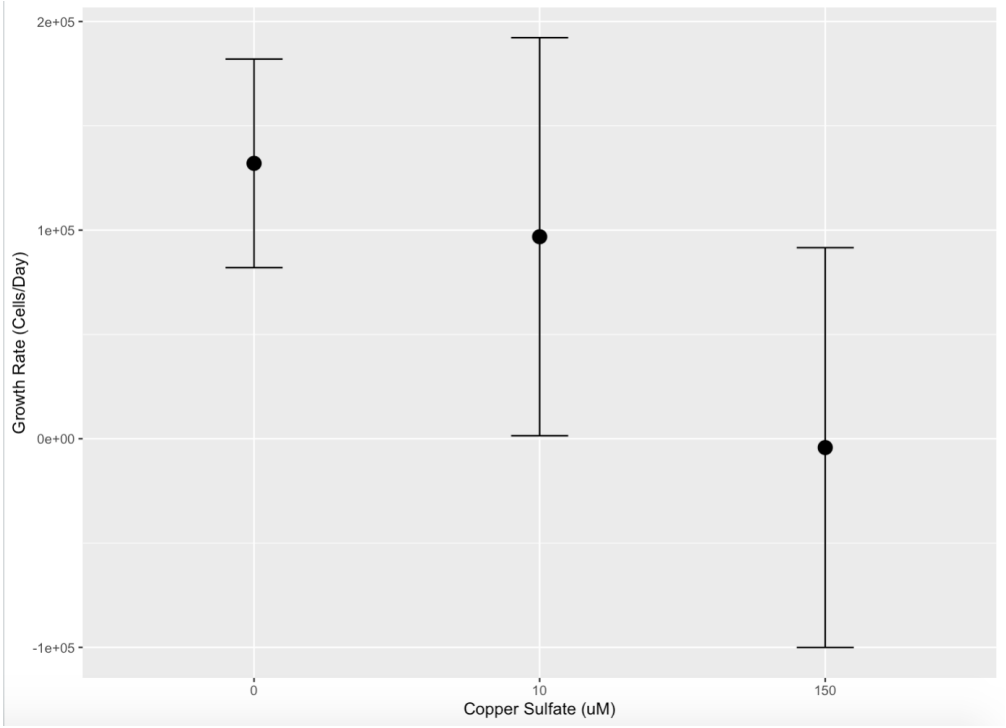

Figure 1: Growth Rate (Cells/Day) in different copper sulfate concentrations (μM). Vertical bars represent the standard deviation of the growth rate under each condition. The circles indicate the mean growth rate of each treatment group (n = 3 at each concentration). The single-factor ANOVA and Kruskill-Wallis tests obtained p-values of 0.09055 and 0.06081.

Moreover, it was observed that at lower copper concentrations, the test tubes which housed the *C. reinhardtii* were clearly greener than those at higher concentrations (Image 2). The difference in colour between the various groups became increasingly more apparent as our study went on, which is a result of the difference in growth rate between the copper sulfate treatments used. Additionally, while counting, it was noted that *C. reinhardtii* cells were smaller in 150μM copper sulfate than the other samples tested. This was also seen to become further propagated by time.

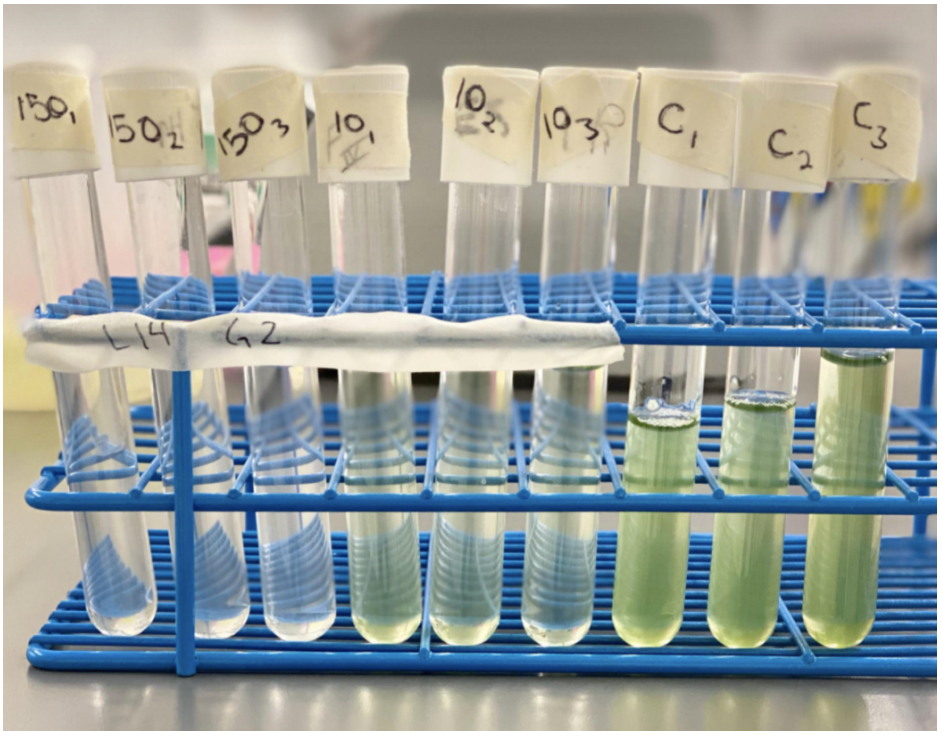

Image 2: Image of test tubes containing *C. reinhardtii* on day 4 of counting (day 6 since the introduction of the microorganism in the test tubes). "C" stands for control (0µM copper sulfate added to media) and the subsequent subscript is the replicate number. 10 and 150 represent 10µM and 150µM copper sulfate concentrations with the subscripts also describing the replicate numbers.

Discussion

Based on the results of our single-factor ANOVA (p-value = 0.09055) and Kruskal-Wallis test (p-value = 0.06081), we fail to reject the null hypothesis of our experiment that copper sulphate concentration will not have an effect on the growth rate of *C. reinhardtii*. Thus, we fail to support our alternative hypothesis that the growth rate of *C. reinhardtii* will be impacted by the concentration of copper sulphate.

This could partly be explained by the large standard deviation in the growth rate for each treatment group. Although we were unable to notice a significant difference in our data analysis, there seemed to be an overall trend in our data where increasing copper sulphate concentrations from 0µM to 150µM negatively impacted the growth of *C. reinhardtii*. The higher growth rate for *C. reinhardtii* in the 0µM and 10µM is likely due to the low concentrations of copper sulphate present. At lower copper sulphate concentrations, copper is beneficial to *C. reinhardtii* as an essential trace metal. This is due to the role copper plays in oxygen-requiring reactions. *C. reinhardtii* absorbs copper from their environments to aid in

electron transfer to oxygen molecules during aerobic respiration. Furthermore, copper plays a role as a cofactor in superoxide dismutase in order to break down harmful oxygen species such as O^{2-}. It is also used as an essential component to produce plastocyanin for *C. reinhardtii*, which is an electron carrier in the process of oxygenic photosynthesis. These beneficial roles of copper in the metabolism of *C. reinhardtii* can potentially explain the higher growth rates found in the concentrations of copper in the 0μM and 10μM treatment levels.

Beyond the 10μM treatment level, we noticed a clear decline in growth rate of *C. reinhardtii*. The decreased growth rate observed at the 150μM can be explained by the high concentration of copper sulphate. Although copper plays a beneficial role in smaller concentrations, higher concentrations of copper can lead to toxicity for *C. reinhardtii* (Jamers et al. 2013). This can lead to the observed lower growth rate at the 150μM treatment level due to copper acting as a heavy metal pollutant at higher concentrations. This leads to copper influencing the growth rate of *C. reinhardtii* involves changes in transcription of oxidative factors. More specifically, excess copper is linked to an increase in the peroxidation of membrane lipids. This increase in oxidation of membrane lipids is believed to reduce photosynthesis and cell growth of cells exposed to higher levels of copper sulfate. In addition, higher copper sulphate levels are linked to decreased levels of antioxidant gene and photosynthesis gene expression. This results in excess reactive oxygen species which can cause oxidative stress. The oxidative stress that results from copper toxicity likely results in decreased cell growth and photosynthesis of *C. reinhardtii* (Jiang et al. 2016). This is supported by the fact that the increased concentrations of copper sulfate resulted in lower growth rate and smaller cells (especially at 150μM).

There are a number of sources of uncertainty and variation. Some examples include different abiotic factors that may influence growth rate, errors when mixing contents in the Eppendorf tube prior to using the haemocytometer to count the cells. The first source of uncertainty and variation can greatly influence the growth rate of the cells as it introduces extraneous variables that can increase or decrease the growth rate. In addition, differences in mixing prior to cell counting can skew results by altering the amount of cells present in the counting zone (150 boxes). The variation and uncertainty in the results were supported by the large standard deviation of growth rate across all experimental groups (especially 10μM and

150μM). One way to improve this experiment is to add additional treatment groups so we can better see the relationship between copper sulfate concentration and growth rate.

Conclusion

As the data in our experiment yielded a p-value > 0.05, we fail to reject the null hypothesis that growth rate will be the same across all experimental groups. Despite this, we established a general negative correlation between copper sulfate concentration and growth rate of C. reinhardtii. This trend can be explained by the fact that increased copper concentration leads to more oxidative stress on the cell which negatively impacts photosynthesis and thus growth rate of C. reinhardtii. Thus, we should try to limit copper contamination in aquatic systems to limit these negative effects on organisms such as C. reinhardtii.

Acknowledgements

We would like to thank our course instructor, Celeste Leander, for providing guidance, advice, and support throughout this experiment. Celeste educated us on the dangers to local salmon and inspired the motivation behind this paper. Additionally, a special thank you to teaching assistants Melanie Warren and Tessa Blanchard for their flexibility and willingness to help in the lab. We would also like to thank the lab technician, Mindy Chow, for ordering supplies, advising on experimental methods, and providing guidance on lab procedures. Lastly, we would like to acknowledge that the study was conducted at the University of British Columbia (Vancouver) which is located on the traditional, ancestral, and unceded territory of the xʷməθkʷəy̓əm (Musqueam) people.

References

Harris, E. H., Stern, D. B., & Witman, G. B. (2009). *The chlamydomonas sourcebook*. Elsevier.

Jamers, A., Blust, R., De Coen, W., Griffin, J. L., & Jones, O. A. H. (2013). Copper toxicity in the microalga Chlamydomonas reinhardtii: an integrated approach. *BioMetals*, *26*(5), 731–740. https://doi.org/10.1007/s10534-013-9648-9

Jiang, Y., Zhu, Y., Hu, Z., Lei, A., & Wang, J. (2016, July 9). *Towards elucidation of the toxic mechanism of copper on the model Green Alga Chlamydomonas reinhardtii*. Ecotoxicology. Retrieved October 19, 2021, from https://link.springer.com/article/10.1007/s10646-016-1692-0.

McCandless, R. G. (1915). Ending Pollution at the Britannia Copper Mine. https://doi.org/10.4095/103819

Rensel, J. E., Haigh, N., & Tynan, T. J. (2010). *Fraser river sockeye salmon marine survival decline and harmful blooms of Heterosigma akashiwo*. Harmful Algae, *10*(1), 98–115. https://doi.org/10.1016/j.hal.2010.07.005

Sasso, S., Stibor, H., Mittag, M., & Grossman, A. R. (2018). From molecular manipulation of domesticated *Chlamydomonas reinhardtii* to survival in nature. *eLife*, *7*, e39233. https://doi.org/10.7554/eLife.39233