The Effects of Copper on *Euglena gracilis*' Oxygen Production

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Abstract

Understanding the effects of heavy metals such as copper on various organisms in an ecosystem is essential to protecting their habitats in an efficient manner. The following study was conducted to measure the oxygen production of the primary producer *Euglena gracilis* in various copper concentrations. Fifteen samples of *E. gracilis* were left in an incubator for a span of three hours in various copper concentrations. Cell count as well as oxygen production was measured previous to, and after incubation and recorded. Even though it was expected that the highest oxygen production would be from samples with lowest copper concentrations, it was found that the treatment with 0.15 mg/L copper concentration had the greatest median for oxygen production. Both 0.15 mg/L and 1.5 mg/L treatment types showed greater oxygen production in comparison to the control group. However, it was concluded that there is no significant correlation between various copper concentrations and oxygen production by *E. gracilis*.

Introduction

Mining of heavy metals was and still is undoubtedly an essential contributor to the worldwide rapid industrialization [6]. Since the 1800s, the productivity growth of copper has continuously increased, with the demands for fast and efficient technology [13]. Copper is a versatile metal, which is primarily used in the manufacturing sector such as electronics, construction, transportation, and information technology [13,14]. However, the consequences of mining these heavy metals are found to be detrimental to not only the environment but to humans as well [7, 11, 16, 17, 18]. Mines produce large amounts of waste during the process of smelting and refining these heavy metals [5]. These mine waste include gaseous and particulate matter emissions, as well as sewage waters, and solid wastes which can be dispersed downstream and further contaminate the surrounding area [10]. Heavy metals, including copper, are non-biodegradable so they accumulate in the environment and are subjected to bioaccumulation and biomagnification, threatening the entire ecosystem [2]. Copper is also an essential heavy

metal used by organisms as a cofactor for several oxidative stress-related enzymes that are crucial for hemoglobin formation, carbohydrate metabolism, catecholamine biosynthesis, and many more [8, 15]. However, excessive exposure to copper can lead to cellular damage and disruption of these important biological functionings [8, 15]. In humans, copper mining activities have been associated with multiple health issues, such as liver cirrhosis, renal failure, and even cancer [7, 11, 18, 17, 18]. With the various negative repercussions of heavy metal mining, it is important to research the consequential effects and analyze the long-term effects as a means to find a solution for mitigation.

Britannia Mine is situated in British Columbia, Canada and was once considered one of the biggest sources of metal contamination into waterways in North America [3]. Between 1898 until 1974, 4 to 40 million litres of acid mine drainage was discharged into Howe Sound depending on the time of the year [1]. Although the mine has been closed for more than 40 years, the copper levels in Howe Sound (~0.15mg/L) is still well over the water quality guidelines (WQG = 0.003mg/L) [3]. These heavy metal contaminations showed adverse effects on the ecosystem with multiple organism growth and reproduction impairments, disturbing the balance of the ecosystem [1]. Copper contamination also has important implications to the First Nation communities situated near the mine, as they rely on the salmon and seafood from Howe Sound as their source of sustenance [1]. This places the First Nation population at greater risk for serious disease and health conditions as a result of the consumption of these contaminated seafood, as it bioaccumulates and biomagnifies throughout the food web [1].

This experiment aims to understand the correlation between copper levels and oxygen production of the *E. gracilis,* a unicellular organism that plays a crucial role as a primary

producer and decomposer in an ecosystem. This is of relevance with regards to the copper contamination of Britannia Mines in British Columbia, Canada and their effect on the ecosystem.

Our research question is how the addition of copper heavy metal to the *E. gracilis* environment affects their oxygen production. We predicted that the addition of copper heavy metal to the *E. gracilis* environment will result in a significant decrease in oxygen production. This is because copper exposure on *E. gracilis* leads to an accumulation of these metals in the mitochondria resulting in oxidative stress [12]. Furthermore, copper is shown to damage photosystem II reaction center rendering it ineffective, so oxygen production can not occur [12].

Methods

Copper medium concentrations were chosen to represent the current situation of Howe Sound as a result of Britannia mine contamination. Copper sulfate pentahydrate ($CuSO4•5H2O$) was used as the copper source, along with mature *E. gracilis* in heterotrophic medium (Recipe from UTEX Culture collection of Algae). Control was established as a negative control, with 0 mg/L of Cu, and 150mL of standard medium was taken from the 500mL stock. Simple dilution calculations were used to measure out 150mL of final Cu concentration of 0.15mg/L and 1.5mg/L for treatment 1 and 2, respectively from a 70mL of a concentrate of $CuSO4•5H2O$. The final volumes of each treatment group equated to 300mL, containing 150mL of the *E. gracilis* culture and 150mL of standard medium. Five samples of 27mL vials were filled to the brim for each treatment, resulting in a total of 15 samples. Oxygen contents were then measured and recorded in each vial using an oxygen probe. Subsequently, cell counts were conducted by using a hemocytometer as well as a microscope. 10 μL of fixative and 100 μL of the sample was used to prepare the hemocytometer. The sample was collected from the spillage that resulted from

closing the filled 27mL vials, so as to not have any air pockets. Only cells present on the top and left visible quadrants were counted for consistency between samples. The labelled samples were then placed into a tray and moved to a 25˚ C incubator with a twelve hour day-light cycle for three hours. Light intensity was measured as 310 lux. After removal of the samples from the incubator, oxygen levels were immediately measured for each vial using the oxygen probe, and a cell count was conducted again.

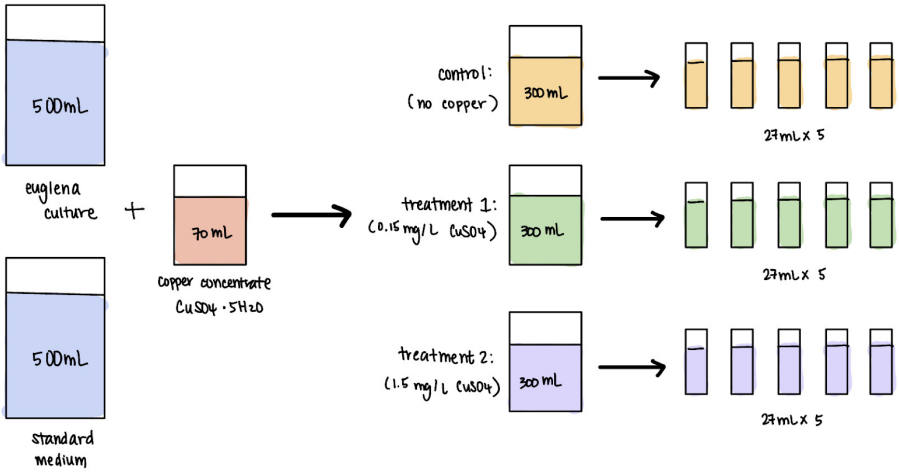

Figure 1: Schematic of the copper concentration preparations for the treatment groups. *N_{total} = 15 and N=5 for each treatment group. Control was made with no copper concentrate added, treatment 1 with a concentration of 0.15mg/L, and treatment 2 with 1.5 mg/L.*

In order to analyze the data, the difference between the oxygen levels per cell after incubation and before incubation were calculated for each vial to obtain the net oxygen production. The programming language "R" was then used to conduct an analysis of variance statistical test on the collected data to find the difference between the means of the three independent treatments, and therefore observe the difference between the oxygen production of

the *E. gracilis* in mediums with various copper concentrations. The means of the change in cell count for each treatment was also analyzed using one-way ANOVA.

Results

Copper concentrations for the control, treatment one and two were 0 mg/L ,0.15mg/L, and 1.5mg/L respectively. As seen in Figure 1, the highest median for the net oxygen production was seen in treatment group 1. Both treatment 1 and 2 showed a higher average net oxygen production per cell than the control. The P-value was calculated to be greater than 0.05, with a value of 0.313. Average final cell counts decreased in respect to initial cell counts for each group. The change in cell count before and after incubation was also found to be statistically insignificant between the three treatment groups with a p = 0.505. The change in cell count was greatest in treatment 1 and lowest in treatment 2.

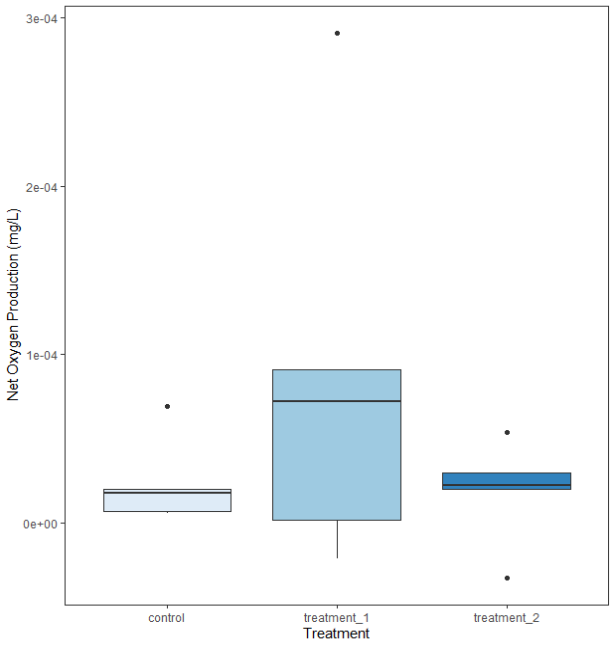

Figure 2: Box plot comparison of measured net oxygen production (mg/L) per cell with various copper concentrations in the treatment groups. Net oxygen production was measured at control (0 mg/L Cu), treatment 1 (0.15mg/L), and treatment 2 (1.5mg/L) per cell, with N=5 for each treatment. Statistical analysis performed by ANOVA. p>0.05.

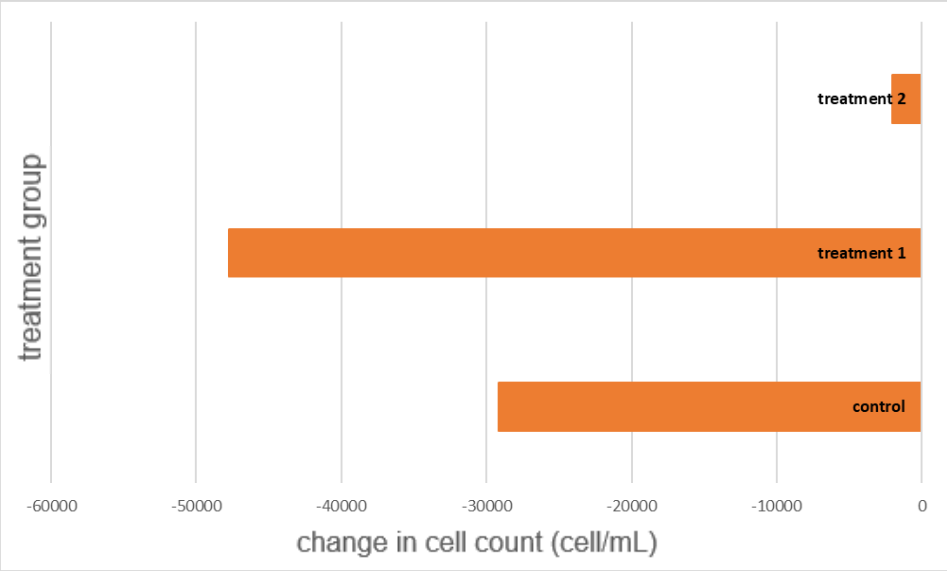

Figure 3: Change in cell count for each treatment group (cells per mL). *Initial count was done prior to incubation (t=0), and final was conducted immediately after (t=3). N=5 for each treatment.* Statistical analysis performed by ANOVA. *p>0.05*

Discussion

In this study, we aimed to determine whether there was a statistically significant effect of copper levels in water, to the *E. gracilis* species. A one way ANOVA test was conducted to understand our findings. The null hypothesis was that there is no significant difference between the amount of oxygen produced by the *E. gracilis* in different copper concentrations. In contrast, the alternative hypothesis was that there is a significant difference between the amount of oxygen produced by the *E. gracilis* in different copper concentrations. The P-value derived from the statistical test is 0.313, which is greater than the significance value of 0.05. Due to this result, we do not reject the null hypothesis which concludes that the differences in oxygen production of the *E. gracilis*, in different copper concentrations given in the treatments, are not significant. In other words, change in copper concentration does not have a significant effect on oxygen production of *E. gracilis*. Since the null hypothesis was not rejected, further statistical analysis was not

conducted in order to understand which treatments vary. The results show that there was a decrease in cell count and possibly an inhibition in cell proliferation. The *E. gracilis* used had switched from heterotrophic to autotrophic, which means it is able to produce oxygen rather than consume it. This was very different from what was expected when a heterotrophic medium was used to culture the *E. gracilis*. The results can be applied to understand that the *E. gracilis* is not affected by the copper contamination in the Britannia Mines. This would mean that the photosystem of the *E. gracilis* is not damaged by the copper, and is still functional. As well, the p-value for the one way ANOVA conducted to understand the mean difference in the cell counts between the treatments was larger than the significance value of 0.05. Therefore we do not reject the null hypothesis of no difference between the mean cell count between the 3 treatments, and conclude that there is no significant difference between the mean cell count of the groups.

In a research paper by Lamas et al., (2002), the effects of copper toxicity on the *E. gracilis* and the accumulation in eukaryotic cells was studied. The study was conducted to test the hypothesis that the presence of vacuoles and the increased number of inclusions in cells that are exposed to metal are used by the *E. gracilis* as a detoxification pathway. This would explain the species not being affected much by the copper as well. The *E. gracilis* uses this pathway to prevent cell damage and keep the metal in specific vacuoles to inhibit its heavy metal effects. To confirm this hypothesis, the metal content of the inclusions was calculated and the results in fact support the hypothesis. The *E. gracilis* vacuoles are involved in the mechanism of detoxification.

To further confirm results, another study found that 0.02mg/L to 2.0mg/L of copper have stimulated photosynthetic efficiency [4]. The *E. gracilis* was treated for seven days with a range of copper concentrations. It was clear that the *E. gracilis* might have a high capacity of adapting to copper stress. The *E. gracilis* seem to take up the copper from the environment and into their

bodies. The paper implies and provides reasoning to show that the *E. gracilis* can be used as a treatment for copper contamination in water, while still providing oxygen for the ecosystem. This also states just how important they are, and so they can speed up the recovery process of the ecosystem.

Further studies we can do to expand our knowledge on the Euglena species and copper effects would be to observe the effects the heavy metal has on heterotrophic versus autotrophic *E. gracilis*. Another would be testing the effect of copper on *E. gracilis* with constant light in a long term study. Moreover, a potential reason why we failed to reject the null hypothesis in this experiment was because there was not a long enough incubation time and the sample size was too small to observe significant changes.

Furthermore, there were a few sources of uncertainty; one being that none of the samples were shaken or swirled before the cell count which could have skewed the results. We may have gotten a fair sample size just from taking the sample from the top. The cell count was conducted from spillage and not the actual vial which might have led to a source of uncertainty as well. Another source of error would be how after removing the samples from the incubator, it took time to count cells and some were counted before others. That caused a variation in time. That also applies for the oxygen measurements.

Conclusion

Overall, the findings did not support the hypothesis that *E. gracilis* is affected by various levels of copper in the environment. No significant correlation was found between copper concentration levels and oxygen production. Such information will be beneficial in order to provide policy makers with an option for a species that is not affected by copper contamination

and may be able to even aid in treatment of copper toxicity in an ecosystem while still providing oxygen. This research project allowed us to add emphasis to the importance and power of the *Euglena gracilis* species.

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