Effect of Copper Sulfate on the Change in Speed of *Caenorhabditis elegans*

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Abstract

Changes in the behaviour of the free-living nematode *Caenorhabditis elegans* are believed to be an effective indicator of toxic exposures. Copper is a known neurotoxin of *C. elegans* when consumed in excess amounts (Gaggelli *et al.* 2006). This experiment aimed to evaluate the change in the rate of movement of *C. elegans* after exposure to different concentrations of copper sulfate. The nematodes were expected to show a greater decrease in speed after being in contact with a more concentrated $CuSO_4$ solution. A drop of the solution was delivered to a single nematode on an agar plate. The nematode was submerged in the solution for a short period of time and its speed was measured before and after exposure. Introducing the nematodes to distilled water showed an average decrease in speed of 73 μm/s after treatment. When exposed to a 0.06 mM $CuSO_4$ solution, the average decrease in speed was found to be 93 μm/s, and after treatment with a 0.46 mM $CuSO_4$ solution, the average decrease in speed was found to be 64 μm/s. At each concentration of treatment solution, the speed of the nematodes decreased following exposure to the $CuSO_4$ solution. However, there was no significant difference in the decrease in speed for the three different treatments. Although excess amounts of copper are known to cause nerve damage in *C. elegans* (Hedges 2010), the exposure time in this experiment may not have been long enough to cause such an effect.

Introduction

Caenorhabditis elegans is a free-living, soil-dwelling nematode with a simple nervous system. With only 302 neurons, its behaviours are limited to a few simple motions, such as eating and moving forward and backward in a sinusoidal fashion (Dhawan *et al.* 2000). It has been noted that many toxic chemicals affect the behaviour of *C. elegans* at concentrations below those that cause physiological changes (William *et al.* 1990). In particular, copper is a metal that, while vital for the organism in trace amounts, can cause damage to neurons when exposure levels are high (Hedges 2010). In humans, copper

toxicity has been linked to neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, although the specific mechanism responsible for this is not known (Hedges 2010). Given that *C. elegans* is a model eukaryote that shares many common molecular and cellular structures with higher organisms, studies can be done with this organism to further our understanding of humans.

The object of this study is to investigate the effect of exposure to increasing copper concentrations on the locomotion of *C. elegans.* Based on a study by Hedges (2010), it is predicted that exposure to this metal will cause impaired movement of the nematodes. By comparing the rate of movement before exposure to the rate of movement after exposure, the change in speed can be compared at different treatment levels. The exposure to copper in this experiment involves submerging the nematodes in a solution of copper sulfate and comparing their speed beforehand to their speed after becoming freed from the solution.

Behaviour is a better indicator of neurotoxicity in *C. elegans* than morphology at low concentrations of toxins (William *et al.* 1990); therefore their movement was measured after treatment at low concentrations of copper sulfate. The results from three different treatments (distilled water, 0.06 mM $CuSO_4$, and 0.46 mM $CuSO_4$) were compared to evaluate the effect of copper exposure on the rate of movement of the nematodes. These concentrations were chosen based on a study by Dhawan *et al.* (2000) that found significant changes in the movement of the nematodes after exposure to similar concentrations of copper.

It is hypothesized that an increase in the concentration of $CuSO_4$ to which *C. elegans* is exposed will cause a decrease in the speed of the nematodes after treatment. . The null

hypothesis is that an increase in the concentration of $CuSO_4$ to which *C. elegans* is exposed will cause an increase or no change in the speed of nematodes after treatment.

Methods

Sterile technique was used throughout the experiment. Kyowa dissecting microscopes were used at 10X magnification, and DinoXcope version 1.4 microscope cameras were used for capturing videos of *C. elegans*. To set up our apparatus, the DinoXcope camera was put in place of the right ocular lens of the microscope. Using the DinoXcope software, a picture of a ruler with 1 mm divisions was taken to measure the field of view for later calibrations of the measurements (Figure 1).

There were three treatments for our experiment. We used distilled water as our control and copper sulfate solutions at concentrations of 0.06 mM and 0.46 mM as our treatment solutions. There were eight replicates for each treatment.

The nematodes we used for this experiment were *C. elegans* N2 wild-type. We transferred enough nematodes for all the replicates onto a clean transfer plate using a nematode pick and the dissecting microscope. This step was performed to separate the adult nematodes from the eggs and larvae. Moreover, it helped us pick out which nematodes were healthy and actively moving. We then took a single adult nematode and placed it in the center of a clean food plate, onto the *E. coli* food source. Following this, we took a 20 second video of the nematode using the DinoXcope camera. This video was used to measure the speed of each nematode prior to treatment.

Making sure the nematode was surrounded by at least 1 mm of *E. coli* without dents in the agar medium, we pipetted 3 µL of treatment solution on top of the nematode

so that it was fully immersed in that drop of solution. If the nematode had moved to the edge of the food source, we carefully moved it with a nematode pick to a different location on the *E. coli* since we would not be able to see the trail of the organism on the agar alone. Once the solution had evaporated completely or the nematode had escaped from the droplet, we took a 20 second video of the nematode right away. This video was used to measure the speed of the nematode after treatment.

Using the videos obtained, we measured the path length of the nematode with a string and ruler and recorded the data (Figure 1). We then converted the distance into its actual size using the ruler calibration from the step above.

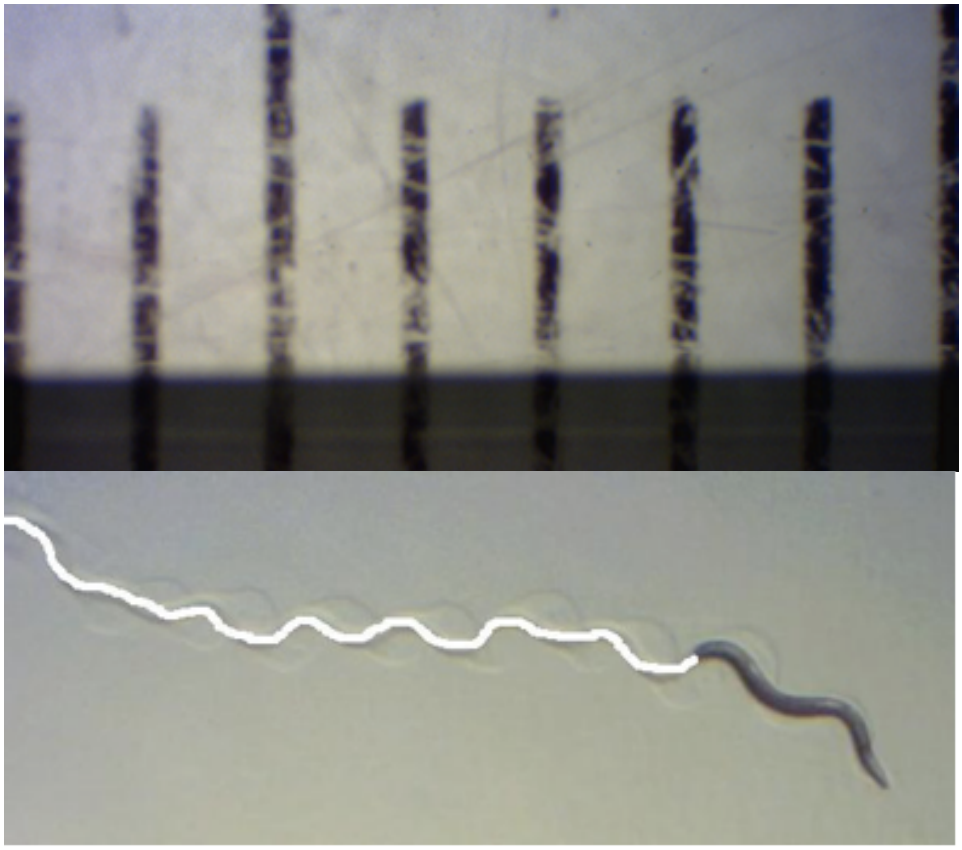

Figure 1. Measuring the distance traveled. Pictures taken under the dissecting microscope at 10X magnification by DinoXcope imaging software. Top: ruler calibration where each division shows 1 mm. Bottom: schematic of the trail of *C. elegans* being measured by a string. The white line represents the string being placed on the video of the nematode.

Finally, we calculated the speed of the nematode by dividing the distance traveled by the time of the video, and found the difference in speed by subtracting the speed after treatment from the speed before treatment. An average was taken for the difference in speed across the eight replicates for each concentration. From those averages, we calculated the 95% confidence intervals for each treatment and compared these to identify whether or not our results were statistically significant.

Results

The results show that every treatment, including the control, caused a decrease in the speed of the nematodes after treatment (Figure 2). For each treatment, the standard deviation was large with respect to the mean change in speed. Figure 2 shows that the confidence intervals of each treatment overlap considerably, each indicating a substantial amount of variation among replicates. There is no observable trend in the data that shows any difference between the results for each treatment. After the nematodes were submersed in distilled water, their speed was found to decrease by 73 μm per second. The nematodes treated with the 0.06 mM copper sulfate solution had a mean decrease in speed of 93 μm per second, and those treated with a 0.46 mM copper sulfate solution had a mean decrease in speed of 64 μm per second. None of the differences between the treatments were statistically significant.

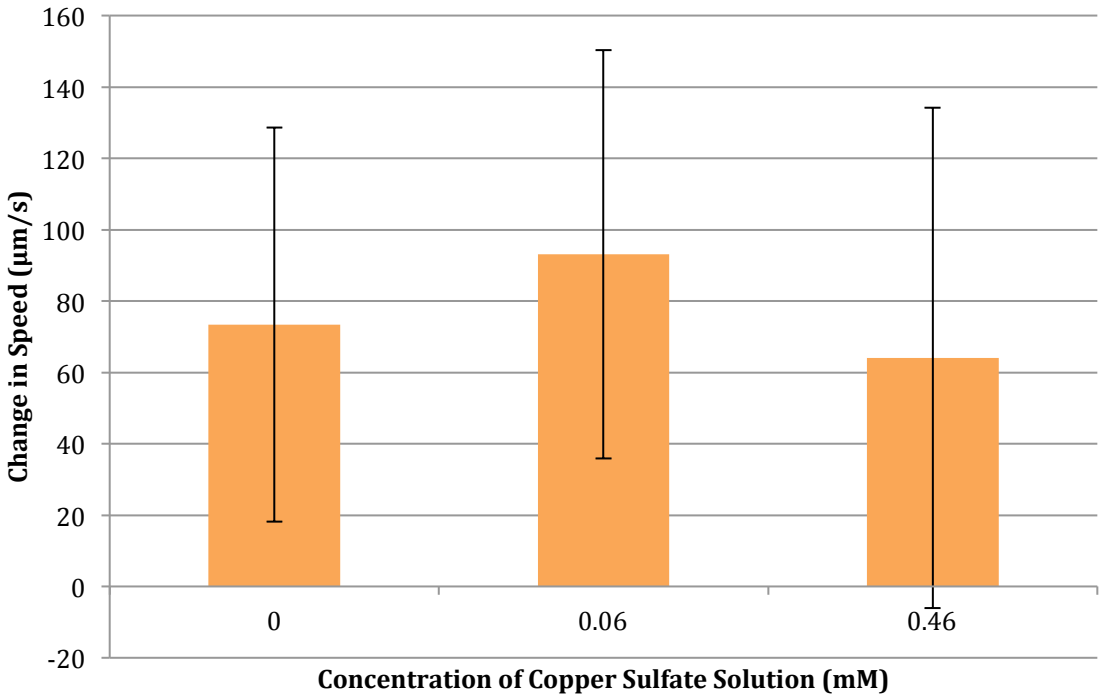

Figure 2. Change in speed of *C. elegans* after treatment with copper sulfate solution versus concentration of treatment solution. Positive values represent a decrease in speed following treatment, and negative values represent an increase in speed following treatment. Values show the mean change in speed (n=8). Change in speed for the distilled water treatment is 73 µm per second, for 0.06 mM, 93 µm per second, and for 0.46 mM, 64 µm per second. Error bars represent 95% confidence intervals.

Qualitative Observations:

Before treatment, the nematodes showed a smooth, sinusoidal movement through the *E. coli* on the agar medium. While submerged in the treatment solutions, they exhibited a rapid thrashing motion as they were attempting to escape. Once they became free from the solution, they returned to their regular sinusoidal motion.

Sample calculations (for concentration of 0.06 mM):

1. Change in speed of *C. elegans* after treatment with 0.06 mM copper sulfate solution:

$$\text{speed before} = \frac{\text{(distance traveled before treatment)}}{\text{(time of travel before treatment)}}$$

$$\text{speed after} = \frac{\text{(distance traveled after treatment)}}{\text{(time of travel after treatment)}}$$

change in speed = speed before - speed after

replicate 1, speed before = 4100 µm/19 s = 215.7894737µm/s
replicate 1, speed after = 2700 µm/19 s = 142.1052632 µm/s
replicate 1, change in speed = 215.7894737µm/s - 142.1052632 µm/s
= 73.68421054 µm/s

2. Mean speed for 8 replicates = $1/8 * \sum_{i=1}^{8}(\textit{change in speed of replicate } i)$

Mean speed at 0.06 mM = 1/8 * (73.68421054 µm/s + (-12.036842 µm/s) + (-10.526316 µm/s) + 94.73684211 µm/s + 94.73684211 µm/s + 126.315789 µm/s + 243.1578947 µm/s + 136.315789 µm/s)
= 93.098026 µm/s

3. Standard Deviation for 8 replicates, s = $\sqrt{\frac{\sum_{i=1}^{8}(x_i-\bar{x})^2}{(n-1)}}$

s = √{[(73.68421054 µm/s – 93.098026 µm/s)^2 + (-12.036842 µm/s - 93.098026 µm/s)^2 + (-10.526316 µm/s - 93.098026 µm/s)^2 + (94.73684211 µm/s - 93.098026 µm/s)^2 + (94.73684211 µm/s - 93.098026 µm/s)^2 + (126.315789 µm/s - 93.098026 µm/s)^2 + (243.1578947 µm/s - 93.098026 µm/s)^2 + (136.315789 µm/s - 93.098026 µm/s)^2]/(8-1)}
= 82.572441 µm/s

4. 95% confidence interval = 1.96 * s/√n
= 1.96 * (82.572441 µm/s)/√8
= 57.219782 µm/s

Discussion

Based on the statistical analysis, the null hypothesis that an increase in the concentration of the $CuSO_4$ treatment solution will lead to a decrease or cause no change in the change in speed of *C. elegans* cannot be rejected. In addition, there is no support for the alternate hypothesis that an increase in the concentration of the $CuSO_4$ treatment solution will lead to an increase in the change in the speed of *C. elegans*. No significant difference was found in the change in speed in the 0.06 mM copper sulfate solution, the 0.46 mM solution, and the control solution of distilled water. The data shows that there was no significant effect from using a copper sulfate solution compared to the control sample of distilled water.

In the experiment, it was expected that a decrease in the rate of movement after the 0.06 mM treatment would be seen and that little or no movement after the 0.46 mM treatment would be observed. These results were anticipated, as there was support from a study by Dhawan *et al.* (2000) showing that a concentration of copper between 0.006 mM and 0.06 mM would have a more significant change in movement than concentrations above 0.269 mM, which were found to be lethal in this study. The nematodes in this experiment were exposed to the copper sulfate solution until they escaped the droplet, or the solution evaporated, which was approximately less than one minute. This exposure time may have not been long enough to see a large decrease in the speed of the nematodes.

There are several possible reasons for the unexpected results of the data. Although there was support from literature (Dhawan *et al.* 2000) that there was enough variability between the concentrations that were used, the results did not support this. This could have been due to the nature of the solution that was used. Also, as the experiment

progressed, the dissecting microscope lamp could have increased in temperature over the three-hour period. Thus, the nematodes that were used at the end of the three-hour period were subjected to higher heat intensity. Since *C. elegans* is able to sense temperature changes in its environment (Shu 2003), this could have played a part in their movement, possibly affecting their change in speed.

Trace amounts of copper are vital for *C. elegans*, since the metal serves as an important cofactor in various chemical reactions within its cells. However, being exposed for extended periods of time to strong enough concentrations of copper can damage certain types of neurons in the nematodes (Hedges 2010). In a study done by Hedges (2010), exposure to copper sulfate was found to cause paralysis in *C. elegans*, which was explained by the fact that the metal can block voltage-gated potassium ion channels at neuromuscular junctions. For this reason, a dramatic effect was expected in the movement of the nematodes after exposure to the copper sulfate solutions. However, the concentrations of copper sulfate used in this experiment were lower than those found to cause complete paralysis in Hedges' experiment, and our exposure times were much shorter (less than one minute compared to several hours).

The measurement techniques used were not as precise as those used in other studies. For example, in the study done by Dhawan *et al.* (2000), computers and special software measured distance traveled with high accuracy. The technique of using a string by hand to measure the distance traveled introduced a significant amount of variability in the measurements taken by each group member. In future experiments, computer software could be used to trace the path of the nematodes and thus increase the precision of measurement.

The qualitative observations noted that the nematodes were thrashing rapidly while in the treatment solutions. The decrease in the speed that was seen at all concentrations may have actually been due to the possibility that each nematode exerted the majority of its energy while trying to escape from the solution.

In a future study, it would be beneficial to look at how *C. elegans* reacts while in the copper sulfate solution, instead of after exposure. Possibly, more of a reaction could be seen afterwards by increasing the time spent in the solution. A wider variety of concentrations may also be useful to compare how the nematodes react after being exposed to the solution.

Conclusion

The experiment showed that there was no significant difference in the change in speed of *C. elegans* after exposure to different concentrations of copper sulfate (0.06mM and 0.46mM) and to the control treatment (distilled water). Therefore, the null hypothesis cannot be rejected and no support is provided for the alternate hypothesis that an increase in the concentration of $CuSO_4$ treatment solution will lead to a decrease in the speed of *C. elegans*.

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