Determining the effect of copper sulfate concentration on the swimming speed of *Tetrahymena thermophila*

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Abstract: To test the toxicity of copper sulphate on the speed of the motile protozoan *Tetrahymena thermophila*, various concentrations of copper sulfate solution were added to the organism's growth medium. Cell speed was determined by capturing the organism's movement using a DinoXcope camera, before analyzing the videos with CellTrack and ImageJ software. Copper sulfate concentrations of 1 ppm, 3 ppm, and 5 ppm were used, as well as both medium and distilled water controls. Tracking the speed of generally slow-moving cells showed a trend of *Tetrahymena thermophila* having the lowest speed (0.25 +/- 0.03 mm/sec) at the highest copper concentration (5 ppm). However, when measuring speeds of generally fast-moving cells, there was a trend towards *Tetrahymena thermophila* showing the highest speed (0.35 +/- 0.04 mm/sec) at the highest copper concentration (5 ppm). This particular trend may be due to the fact that toxins are often localized, and when a specific cell is exposed to a high amount of toxin, it tries to escape by swimming faster. Though our results are not statistically significant, we see a trend towards high levels of copper sulfate affecting the organism, with fast-moving cells becoming faster, and slow-moving cells becoming slower.

Introduction

Tetrahymena thermophila is a eukaryotic, unicellular, ciliate protozoan that lives in aquatic habitats. As a highly motile protozoan, it has the ability to use ciliate structures on its surface to move swiftly within surrounding waters (Frankel 2000). This mobility is particularly important for activities such as feeding (Eisenmann *et al.* 1998). However, there is increasing concern over the amount of water contamination by heavy metals coming from sources such as industrial and sewage waste (Ryu *et al.* 2011). Compared to organic pollutants, these heavy metals are especially dangerous as they are not biodegradable (Martín-González *et al.* 2005). The purpose of this study is to assess if the presence one of these heavy metals, copper sulfate, impacts *Tetrahymena thermophila*'s motility. Exposure to heavy metals such as copper has been shown to have profound effects on ciliate protozoans (Ruthven and Cairns 1973). By

investigating *Tetrahymena thermophila* in this experiment,more can be learned about the specific threat posed to the organism's mobility by these growing pollutants.

Once exposed to abnormal, extreme conditions, these organisms have the ability to transform into a slender shape to swim more rapidly (Frankel 2000). However, if the unfavourable conditions become harsh enough, the results can be more severe. Indeed, one experiment found that, at a pH of 8, a copper sulfate concentration of 10 ppm lead to a 0% survival rate after 20 hours (Schlenk and Moore 1994). In our study, a lower range (0 ppm to 5 ppm) of copper sulfate concentration was chosen to ensure that *Tetrahymena thermophila* maintained a relatively high survival rate. This was because we wanted to investigate how various concentrations of copper sulphate could cause the organism's behaviour, not survival rate, to change. For this experiment, the hypotheses are as follows:

H_a: An increase in copper sulfate concentration will lead to a decrease in the motility of *Tetrahymena thermophila.*

H_0: An increase in copper sulfate concentration will lead to an increase or no change in the motility of *Tetrahymena thermophila.*

Since copper sulphate has been shown to negatively affect the growth of *Tetrahymena thermophila* (Schlenk and Moore 1994), it is reasoned that this metal provides a clear stress to the organism, and will also decrease its motility.

Methods

We used *Tetrahymena thermophila* grown in a NEFF medium containing: 0.25% proteose peptone, 0.25% yeast extract, 0.55% glucose, and 33 μM $FeCl_3$ (*Chlamydomonas* stock). Cells were initially allowed to incubate for a little less than a week at room temperature, and were retrieved at a concentration of 15,225 cells/mL.

We used five categories for this experiment – three treatments of different concentrations of $CuSO_4$ and two controls: one using distilled water in place of $CuSO_4$, and the other replacing the $CuSO_4$ with NEFF growth medium. Each treatment had three replicates, which amounted to a total of fifteen replicates.

We used concentrations of 1 ppm, 3 ppm, and 5 ppm $CuSO_4$ for our treatments. We selected these concentrations as our experimental conditions lie within pH 6 – pH 8, and within this range, 1 ppm – 5 ppm $CuSO_4$ concentrations have been shown to have a noticeable stress on *Tetrahymena thermophila*, without killing the population (Schlenk and Moore 1994).

We prepared each of our replicates to have a total volume of 9 mL, doing so in 15 mL test tubes. For this, we first added 8.95 mL of cell culture. This culture was made up of 2.96 mL of *Tetrahymen*a cell stock (which we counted as containing about 45,000 cells) and 5.99 mL of the NEFF growth medium. We then varied the remaining 0.05 mL by category. For the $CuSO_4$ treatments, this 0.05 mL was a mix of distilled water and $CuSO_4$ solution. For the medium and distilled water controls, the 0.05 mL consisted entirely of NEFF growth medium and distilled water, respectively. Each replicate, once prepared, had a concentration of 5000 cells/mL. We chose this concentration as we wished the cells to be concentrated enough to compensate for any lost from $CuSO_4$ treatments, while remaining safely above 750 cells/mL – a critical density under which *Tetrahymena* populations die (Christensen *et al.* 1995).

We were not concerned about having any variation of pH between replicates as it was deemed negligible (C. Pollock, BIOL 342 professor, personal communication), so factors related to pH were not considered in the experiment.

We prepared samples of the replicates on slides after about 24 hours incubation at room temperature (about 21°C), and recorded videos of the cells using a DinoXcope attached to a

compound microscope (see Figure 1). We prepared three slides for each replicate, and each slide was recorded once for a total of 45 videos. We used two computer programs to analyze the videos and recover the *Tetrahymena* speed data. We used the program CellTrack (Sacan *et al*. 2008) to analyse the videos by using the tracking feature of the program (see Figure 2), and as the data was unreliable for fast-moving cells, we also analyzed the videos using the program ImageJ. This program allowed the manual measurement of cell displacement over time between two frames (see Figure 3). We analysed a single cell by each method in each video. With the tracking program we selected the first trackable cell (usually a slow moving cell with a defined outline) that appeared in each video, and with our displacement measures we measured the fastest cell that moved in a straight line. We analysed our data from both methods separately, using 95% confidence intervals to compare between categories of treatments.

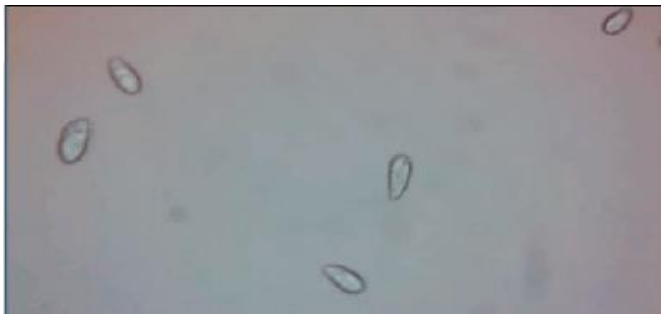

Figure 1. Frame 219 of 445 of a video captured by DinoXcope showing slide 1 of replicate 1 from the distilled water control.

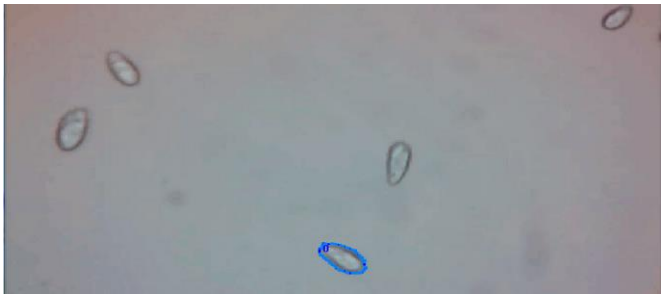

Figure 2. Frame 218 of the video in Fig. 1, showing the use the CellTrack program to track a cell by its outlined cell membrane.

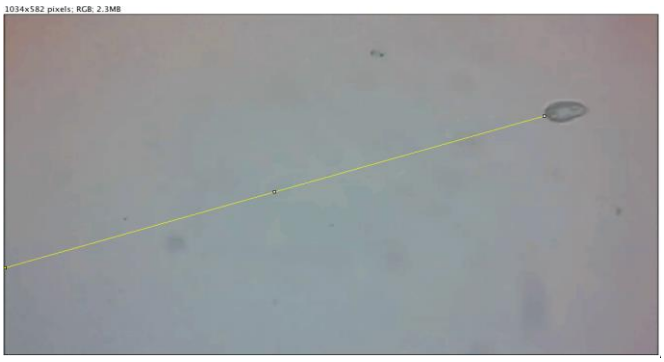

Figure 3. Using ImageJ to determine the distance travelled by a cell.

Results

The *Tetrahymena* speed averages from CellTrack, displayed in Figure 4, indicate that the distilled water control was significantly faster than all other treatments. Indeed, at 0.68 mm/sec (95% CI: 0.61-0.74 mm/sec), it was nearly double the speed of those other treatments. Another significant difference was found between the 1 ppm $CuSO_4$ and 5 ppm $CuSO_4$ treatments, their speeds being 0.34 mm/sec (95% CI: 0.30-0.39 mm/sec) and 0.25 mm/sec (95% CI: 0.22-0.28 mm/sec) respectively. While there were otherwise no statistically significant results, a trend was observed within the $CuSO_4$ containing groups; as the concentration of $CuSO_4$ in the solution increased, the tracked *Tetrahymena* swam

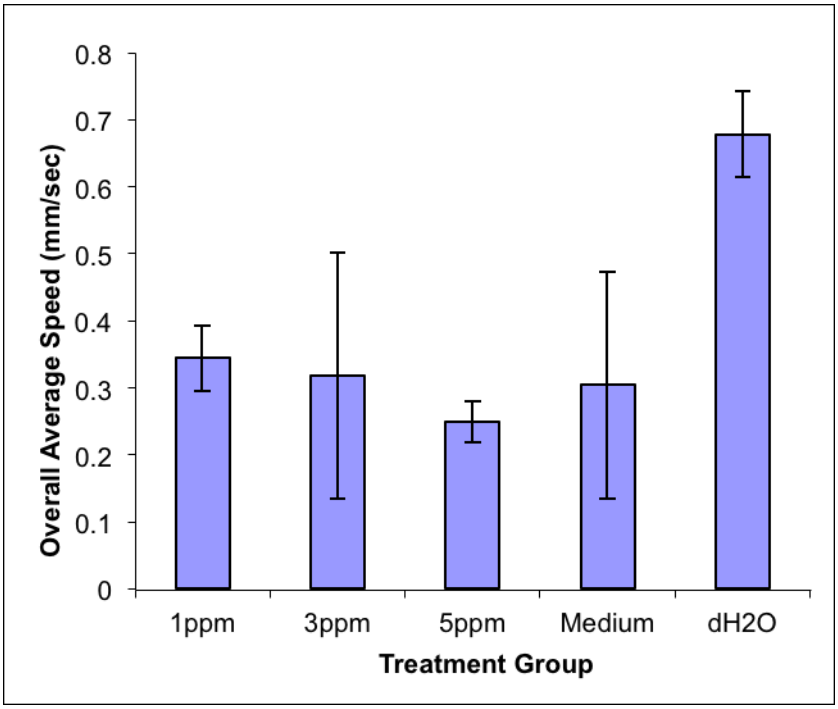

Figure 4. Average speed of *Tetrahymena thermophila* after ~24 hours of exposure to 1 ppm, 3 ppm, 5 ppm $CuSO_4$ concentrations, 0.05 mL distilled water, or only NEFF medium. Error bars show 95% confidence intervals. Cell speed tracked using CellTrack software.

slower, from 0.34 mm/sec, to 0.32 mm/sec, to 0.25 mm/sec at 1 ppm, 3 ppm, and 5 ppm respectively.

In the ImageJ displacement speed data, shown in Figure 5, there was a statistically significant difference between speeds of *Tetrahymena* in the distilled water control (0.41 mm/sec with 95% CI: 0.39-0.42 mm/sec), and both the 3 ppm and 1 ppm $CuSO_4$ treatment solutions – which had speeds of 0.32 mm/sec (95% CI: 0.29-0.34 mm/sec) and 0.27 mm/sec (95% CI: 0.18-0.37 mm/sec) respectively. Although there was a trend among the treatments, this pattern was the opposite of that of the tracked speed data from Figure 4. The displacement data shows faster movement at higher concentrations of $CuSO_4$, ranging from 0.27 mm/sec, to 0.32 mm/sec, to 0.35 mm/sec for 1 ppm, 3 ppm, and 5 ppm respectively.

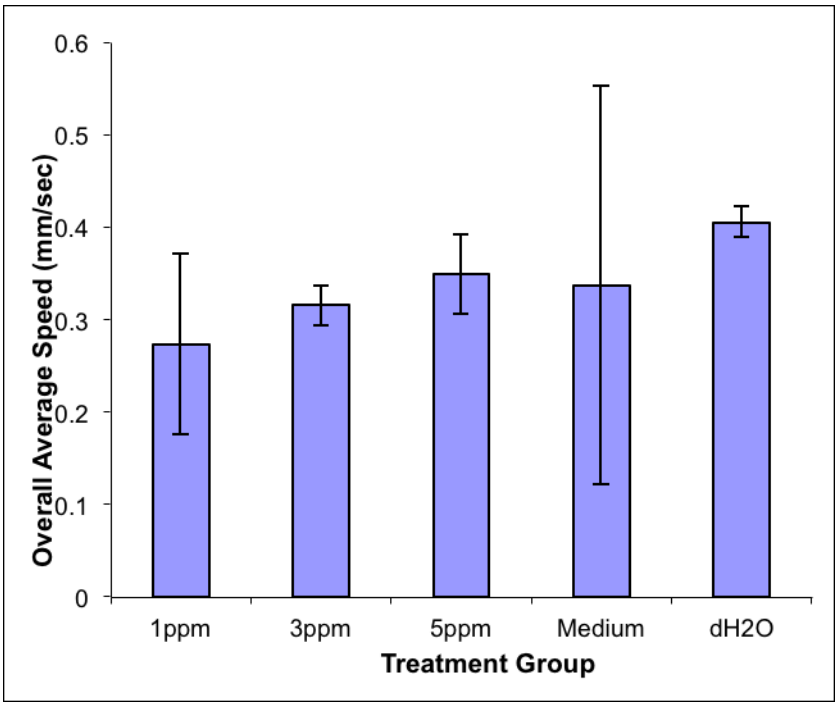

Figure 5. Average speed of *Tetrahymena thermophila* after ~24 hours of exposure to 1 ppm, 3 ppm, 5 ppm $CuSO_4$ concentrations, 0.05 mL distilled water, or only NEFF medium. Error bars show 95% confidence intervals. Cell displacment measured using ImageJ software.

In both methods of speed measurement, the distilled water control cells were the fastest, with this difference being the largest for cells tracked using CellTrack. The cells in the control medium had an unusually high amount of variance – 0.022 for tracked measurements and 0.036412 for displacement measurements. This meant that the medium control never had any significant difference from any treatment, apart from the CellTrack water control.

Sample Calculations:

Cell Track gives average speed in pixels/frame. Data was recorded at 30 frames/sec, and at the magnification used, 165 pixels represented a real size of 0.2 mm:

0.2 mm/165 pixels = 0.00121212 mm/pixel
(Average Speed) pixels/frame × 0.00121212 mm/pixel × 30 frames/sec =
(Average Speed) × 0.0363636 mm/sec

1 ppm, replicate 1 average speed using CellTrack:

Slide 1: 0.173748626 mm/sec
Slide 2: 0.667304387 mm/sec
Slide 3: 0.297296903 mm/sec
Total: 0.173748626 + 0.667304387 + 0.297296903 = 1.138349916
Total/# slides= 1.138349916 / 3 = 0.379449972 mm/sec
Average speed: 0.379449972 mm/sec

1 ppm total average speed using CellTrack:

Replicate 1: 0.379449972 mm/sec
Replicate 2: 0.358612768 mm/sec
Replicate 3: 0.296467291 mm/sec
Average = 0.344843 mm/sec

Variance:
$((1/(3-1)) \times ((0.379449972-0.344843)^2) + ((0.358612768-0.344843)^2) + ((0.296467291-0.344843)^2))$
=0.001863729 mm/sec

95% confidence intervals for 1 ppm using CellTrack:

1.96 × ((Std dev) / S.Root(3))
=0.048852505 mm/sec

ImageJ

Same process, but slide average speeds are given by:
(Distance given by program in mm)/(Time between frames used in sec)

Discussion

Given the large variance in the collected data, we failed to reject our null hypothesis, and found that increased concentrations of copper sulfate solution did not have a significant consistent effect on the motility of *Tetrahymena thermophila*. This may have been due to procedural errors, the first being the erroneous assumption that the introduction of 50 μL of distilled water to 8950 μL of growth medium would not dilute the solution enough to have it affect cell behavior. When placed in an inorganic medium such as distilled water, *Tetrahymena thermophila* responds by making behavioral and structural changes such as decreasing its volume and increasing the number of cilia on its body, to increase its swimming speed (Frankel 2000). We observed an increase in swimming speed in the distilled water control compared to the cell medium control, suggesting that this response might be occurring in our inorganic treatments. However, when videos from the two controls are compared, while cilia cannot be seen and so cannot be commented on, there is no obvious difference in cell shape or size. Still, the addition of water to the medium should be minimized in future experiments.

The methods of data collection used were likely subject to sampling bias. The program CellTrack (Sacan *et al.* 2008) had limitations when combined with the low frame rate of our videos and some variation in video quality. Cells moving too quickly could not be tracked with the program, so when collecting data, slower cells were usually selected. Also, in some videos, low image quality meant that tracking had to be done manually. Therefore, cells were selected based not on speed, but on whether or not the program could identify its shape as a cell, which was a necessary first step in the process. Video quality varied between the treatments, and notably, the distilled water control's videos were all of poor quality. These videos were tracked

manually, which may also explain this control's higher mean speed as it could have introduced a measurement bias.

When cell displacement was used as a measure of speed, it was limited to the fastest cells, specifically those moving in a straight line. Cells making turns must slow down to do so (Levandowsky *et al.* 1984), so this was a reasonable limitation to set. Using this method, we realized that there was a discrepancy among replicates in the NEFF medium control; in replicate three there were no truly fast moving cells. This dramatically lowered the mean speed of this control and accounts for the large error bars.

The tracking method, used to measure the slower cells, demonstrates a trend towards cells in higher copper concentrations moving slower. In normal ecosystems, *Tetrahymena* prey on bacteria (Eisenmann *et al.* 1998) and their typical swimming pattern is rapid movement in a straight line punctuated by sudden changes in direction. The organism swims in search of bacteria, and by increasing its speed while looking for a chemical signal between feedings, it maximizes its grazing efficiency (Levandowsky *et al.* 1988). In our treatments, the cells are suspended in a culture with no bacteria present, but they have no way of knowing this, and so continuing searching behaviour would be expected. It follows, then, that the slower swimming speed observed indicates decreased cell fitness. This is consistent with examinations of the effects of copper toxicity on *Tetrahymena thermophila* mortality. With increases in copper concentrations came decreased fitness and higher death rates (Schlenk and Moore 1994). Cells observed to be slower are likely those more heavily affected by toxicity.

The displacement method, illustrating speeds of the fastest cells, shows an opposing trend; at higher concentrations of copper sulfate cells swim faster. This is consistent with known behavior of *Tetrahymena thermophila* in the presence of a localized toxin. The organism will

exhibit directed movement either away, or in some cases toward, the toxin, with this effect increasing with toxin concentration (Gilron *et al.* 1999). It follows that the same behavior would be displayed even if the toxin was not localized. The cell would increase its speed to try and remove itself from the toxic area, and an increase in concentration of copper sulfate would correspond with an increase in effort. It is also possible, but seems less likely, that the cells were attracted to the copper sulfate. However, these two behaviors are not distinguishable in this experiment. The peak speed of the *Tetrahymena thermophila* exposed to copper sulfate is still below the average speed of those exposed to the distilled water control.

Taken together, these results suggest that *Tetrahymena thermophila* may increase its speed to deal with increased copper sulfate concentrations, but eventually is more heavily impaired by the toxicity. Additional information could be provided by performing experiments measuring the ratio of fast to slow moving cells at varying concentrations of this toxin.

Conclusion

While the sources of error and, specifically, procedural problems in this experiment led to an inability to reject the null hypothesis, there does seem to be a relationship between copper sulfate concentration and the behavior of *Tetrahymena thermophila* that bears more investigation. The trends present in the data suggest that interesting information on this organism's speed, vitality, and behavior could be obtained, but procedures used in this experiment would have to be modified or refined to gain more meaningful information.

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Literature Cited

Christensen, S. T., Wheatley, D. N., Rasmussen, M. I., and Rasmussen, L. 1995. Mechanisms controlling death, survival and proliferation in a model unicellular eukaryote *Tetrahymena thermophila*. Cell Death and Differentiation, **2** (4): 301-308.

Eisenmann, H., Harms, H., Meckenstock, R., Meyer, E., and Zehnder, A. 1998. Grazing of *Tetrahymena* sp. on adhered bacteria in percolated columns monitored by *in situ* hybridization with fluorescent oligonucleotide probes. Applied Environmental Microbiology, **64** (4): 1264-1269.

Frankel, J. 2000. Cell Biology of *Tetrahymena thermophila*. pp. 27-125. In: D. Asai and J. Forney (ed.), Methods in Cell Biology. Academic Press, San Diego, CA.

Gilron, G., Gransden, S. G., Lynn, D. H., Broadfoot, J., and Scroggins, R. 1999. A behavioral toxicity test using the ciliated protozoan *Tetrahymena thermophila*. Environmental Toxicology and Chemistry, **18** (8): 1813-1816.

Levandowsky, M., Cheng, T., Kehr, A., Kim, J., Gardner, L., Silvern, L., Tsang, L., Lai, G., Chung, C., and Prakas, E. 1984. Chemosensory responses to amino acids and certain amines by the ciliate *Tetrahymena*: A flat capillary assay. The Biological Bulletin, **167**: 322-330.

Levandowsky, M., Klafter, J., and White, B. S. 1988. Feeding and swimming behavior in grazing microzooplankton. Journal of Eukaryotic Microbiology, **35** (2): 243-246.

Martín-González, A., Borniquel, S., Díaz, S., Ortega, R., and Gutiérrez, J. C. 2005. Ultrastructural alterations in ciliated protozoa under heavy metal exposure. Cell Biology International, **29** (2): 119-126.

Ruthven, J. A., and Cairns, J. 1973. Response of fresh-water protozoan artificial communities to metals. Journal of Eukaryotic Microbiology, **20** (1): 127-135.

Ryu, J., Khim, J. S., Kang, S., Kang, D., Lee, C. Koh, C. 2011. The impact of heavymetal pollution gradients in sediments on benthic macrofauna at population and community levels. Environmental Pollution, **159** (10): 2622-2629.

Sacan, A., Ferhatosmanoglu, H., and Coskun, H. 2008. CellTrack: An open-source software for cell tracking and motility analysis. Bioinformatics, **24** (14): 1647-1649.

Schlenk, D., and Moore, C. T. 1994. Effect of pH and time on the acute toxicity of copper sulphate to ciliated protozoan *Tetrahymena thermophila*. Bulletin of Environment Contamination and Toxicology, **53** (6): 800-804.