**Copper-Washed Soil Toxicity and the Aquatic Arthropod *Daphnia magna*: Effects of Copper Sulfate Treatments**

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## INTRODUCTION

Copper is a heavy metal which can be toxic to aquatic organisms at high concentrations. For this reason, copper sulfate has been used to treat algal blooms and invertebrate populations in residential ponds. However, there are detrimental environmental implications. Our research was motivated by the idea that copper could leach into the groundwater or be carried into a nearby lake or stream during a rainstorm. This transport could cause contamination in natural waters and create toxic soils in these natural systems. Investigation of the effects of this contamination on the soil and benthic organisms as well as pelagic organisms would then become important. Our study involved determining the amount of copper adsorbed by the soil by viewing the effects of the toxic soil on the survival rates of *Daphnia magna*.

The area of study is the Lake Macatawa watershed. The three different water systems investigated were a lake (Kollen Park), a pond (Outdoor Discovery Center), and a creek (Pine Creek). Kollen Park was a former city landfill and Lake Macatawa is directly accessible through the park. Outdoor Discovery Center is a wildlife preserve which had one pond treated approximately 15-20 years ago, but we made sure to avoid this pond for our samples. Finally, Pine Creek samples were taken near the fork of the river just off the nature trail. These places were tested for copper and found to have negligible concentrations. Therefore, these sites were ideal for copper toxicity testing. The Kollen Park soils contained much larger grain sizes than the Outdoor Discovery Center and Pine Creek. It was, therefore, assumed that clay concentrations were higher in the Pine Creek and Outdoor Discovery Center samples as opposed to the Kollen Park samples.

## METHODS

*Soil Toxicity*

Soils were collected in buckets from the three sample sights. They were returned to the lab and dried in a drying oven set at 100ºC for approximately 24 hours or until completely dry. Large rock fragments, glass, plastic, leaves, and twigs were removed using standard engineering sieve #10. Dried soils were then added to 20 mL disposable scintillation vials (8 mL per vial). Copper concentrations of 0 ppm (control), 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, 100 ppm, and 150 ppm were prepared in RO $H_2O$. 10 mL of the appropriate copper concentration were added to each vial and allowed to settle for 24 hours. The total number of vials tested for each concentration was 12 (4 vials per location for 3 locations). After 24 hours, the copper solutions were decanted and the decant was saved. The soils were rinsed with 15 mL of dechlorinated water. The rinseate was decanted and saved. Another 5 mL of dechlorinated water was added to each vial. Then, 5 lively *Daphnia magna* were added to each vial. The lid was loosely placed on the vials and they were stored in a drawer for a period of 24 hours. Once the 24 hour cycle was completed, the number of live and the number dead were counted and recorded. Analysis of the data was completed using a G-test which is more accurate and appropriate for our data when compared to a $X^2$ analysis.

*AA-Atomic Absorption Spectroscopy*

The absorbance of the copper was measured at 324.4 nm all other parameters were set to default settings. The AA was calibrated using standard copper solutions of 10, 20, 30, 40, and 50 ppm. The concentration was then plotted against the absorbance and a linear regression was

conducted. The decanted copper solution and the rinseate collected were then tested on the AA. Their concentrations were determined from the absorbance according to the regression line created from the copper standards.

## RESULTS

Results from all the vials were compiled and the totals were used as the actual values. Using the row and column totals, the expected values were determined.

| *Kollen Park* | | | | | | |
|---|---|---|---|---|---|---|
| | *ACTUAL* | | | *EXPECTED* | | |
| **[Cu]** | **Alive** | **Dead** | **Total** | **Alive** | **Dead** | **Total** |
| **0** | 41 | 6 | 47 | 36.3 | 10.7 | 47 |
| **10** | 27 | 0 | 27 | 20.9 | 6.1 | 27 |
| **20** | 46 | 16 | 62 | 47.9 | 14.1 | 62 |
| **30** | 21 | 8 | 29 | 22.4 | 6.6 | 29 |
| **40** | 19 | 4 | 23 | 17.8 | 5.2 | 23 |
| **50** | 28 | 17 | 45 | 34.8 | 10.2 | 45 |
| **100** | 12 | 8 | 20 | 15.5 | 4.5 | 20 |
| **150** | 17 | 3 | 20 | 15.5 | 4.5 | 20 |
| **total** | 211 | 62 | 273 | 211 | 62 | 273 |

Table 1. Data collected for Kollen Park and used to determine the G-value according to the G-test analysis.

| *Outdoor Discovery Center* | | | | | | |
|---|---|---|---|---|---|---|
| | *ACTUAL* | | | *EXPECTED* | | |
| **[Cu]** | **Alive** | **Dead** | **Total** | **Alive** | **Dead** | **Total** |
| **0** | 41 | 9 | 50 | 38.4 | 11.6 | 50 |
| **10** | 27 | 5 | 32 | 24.5 | 7.5 | 32 |
| **20** | 38 | 9 | 47 | 36.1 | 10.9 | 47 |
| **30** | 23 | 7 | 30 | 23.0 | 7.0 | 30 |
| **40** | 20 | 3 | 23 | 17.6 | 5.4 | 23 |
| **50** | 33 | 9 | 42 | 32.2 | 9.8 | 42 |
| **100** | 20 | 4 | 24 | 18.4 | 5.6 | 24 |
| **150** | 12 | 19 | 31 | 23.8 | 7.2 | 31 |
| **total** | 214 | 65 | 279 | 214.0 | 65 | 279 |

Table 2. Data collected for the Outdoor Discovery Center and used to determine the G-value according to the G-test analysis.

| *Pine Creek* | | | | | | |
|---|---|---|---|---|---|---|
| | *ACTUAL* | | | *EXPECTED* | | |
| **[Cu]** | **Alive** | **Dead** | **Total** | **Alive** | **Dead** | **Total** |
| **0** | 35 | 12 | 47 | 37.7 | 9.3 | 47 |
| **10** | 22 | 6 | 28 | 22.5 | 5.5 | 28 |
| **20** | 38 | 13 | 51 | 41.0 | 10.0 | 51 |
| **30** | 23 | 9 | 32 | 25.7 | 6.3 | 32 |
| **40** | 18 | 3 | 21 | 16.9 | 4.1 | 21 |
| **50** | 40 | 4 | 44 | 35.3 | 8.7 | 44 |
| **100** | 19 | 2 | 21 | 16.9 | 4.1 | 21 |
| **150** | 21 | 4 | 25 | 20.1 | 4.9 | 25 |
| **total** | 216 | 53 | 269 | 216 | 53 | 269 |

Table 3. Data collected for Pine Creek and used to determine the G-value according to the G-test analysis.

Once the data was compiled, the G-test equation was used to determine the G-value.

$$G = 2\sum_i O_i \bullet \ln\left(\frac{O_i}{E_i}\right)$$

| | Kollen Park | Outdoor Discovery Center | Pine Creek |
|---|---|---|---|
| G-value | 26.905 | 29.058 | 9.355 |

Table 4. Summary of the G-test analysis. The degrees of freedom is 7 and the critical value ($\alpha$=0.05) is equal to 14.07.

Using these G-test values and a critical value of 14.07 ($\alpha$=0.05), it was determined that both Kollen Park and the Outdoor Discovery Center had significant values because they were above the critical value. However, Pine Creek did not have a statistically significant value. Figure 1 shows that there is a somewhat negative trend in the survival rate as the concentration of the copper wash increases. However, there were discrepancies in the expected $LC_{50}$ and the actual concentration of the wash needed to increase the mortality rate because approximately 50% of the *Daphnia* were still alive even at the 100 ppm concentration wash when the $LC_{50}$ is less than 1 ppm (Orme).

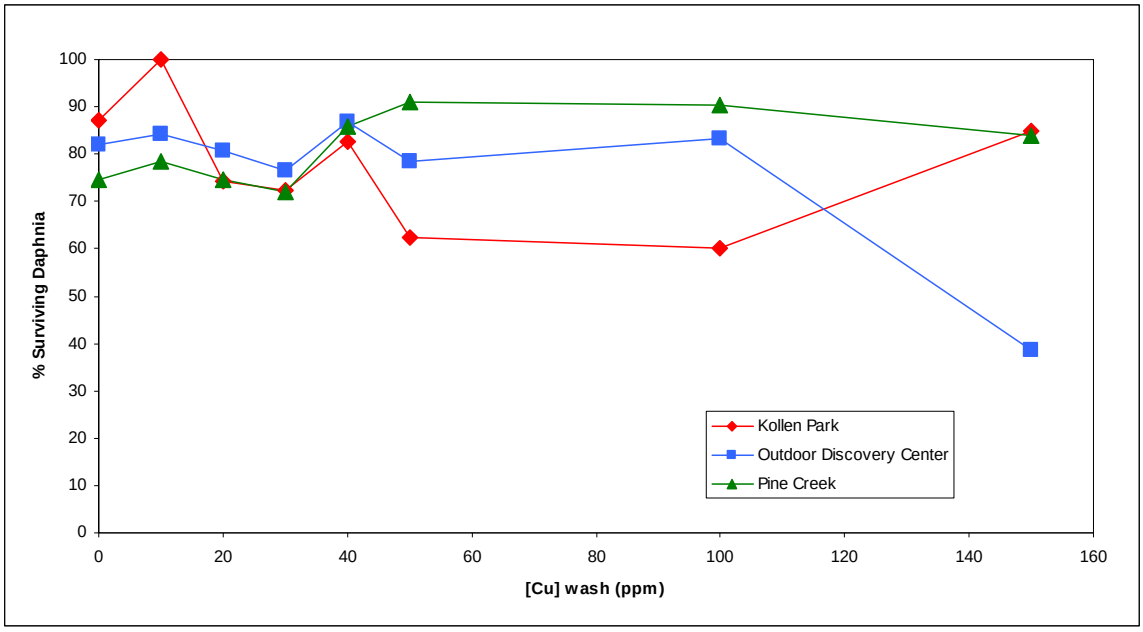


Figure 1. Survival rate of *Daphnia magna* according to our soil toxicity tests.

The AA was run in order to determine the destination of the copper added to the samples. The break down of the total amount of copper added, mg lost in the decanted solution (solution removed after 24 hours of settling), and the amount removed in the rinseate shows that any copper not lost in the decant or rinseate must be left in the soil. Note that there is very little copper that is lost in the rinseate meaning that any copper that had sorbed to the soils remains in the soil and is not easily lost in solution again.

| | | Kollen Park | | | Pine Creek | | | Outdoor Discovery Center | | |
|---|---|---|---|---|---|---|---|---|---|---|
| [wash] (ppm) | Total mg of Cu | mg in decant | mg in rinseate | mg left in soil | mg in decant | mg in rinseate | mg left in soil | mg in decant | mg in rinseate | mg left in soil |
| 0 | 0.0000 | 0.0048 | 0.0158 | -0.0206 | 0.0061 | 0.0172 | -0.0232 | 0.0022 | 0.0034 | -0.0056 |
| 10 | 0.1000 | | 0.0047 | 0.0953 | 0.0404 | 0.0047 | 0.0548 | 0.0392 | 0.0047 | 0.0561 |
| 20 | 0.2000 | 0.1054 | 0.0075 | 0.0871 | 0.0812 | 0.0061 | 0.1127 | 0.0748 | 0.0061 | 0.1191 |
| 30 | 0.3000 | 0.1385 | 0.0103 | 0.1513 | 0.1066 | 0.0075 | 0.1859 | 0.1181 | 0.0075 | 0.1744 |
| 40 | 0.4000 | 0.1741 | 0.0103 | 0.2156 | 0.1423 | 0.0089 | 0.2488 | 0.1385 | 0.0075 | 0.2540 |
| 50 | 0.5000 | 0.2339 | 0.0158 | 0.2503 | 0.1830 | 0.0116 | 0.3054 | 0.1588 | 0.0172 | 0.3240 |

Table 5. Summary of the AA results which accounts for all of the copper added to each sample.

Using the amount of copper left in the soils compared to the amount of copper added reveals the percentage of the copper that was adsorbed to the soils. The Outdoor Discovery Center and Pine Creek have slightly higher fractions of copper that remain in the soils when compared to Kollen Park.

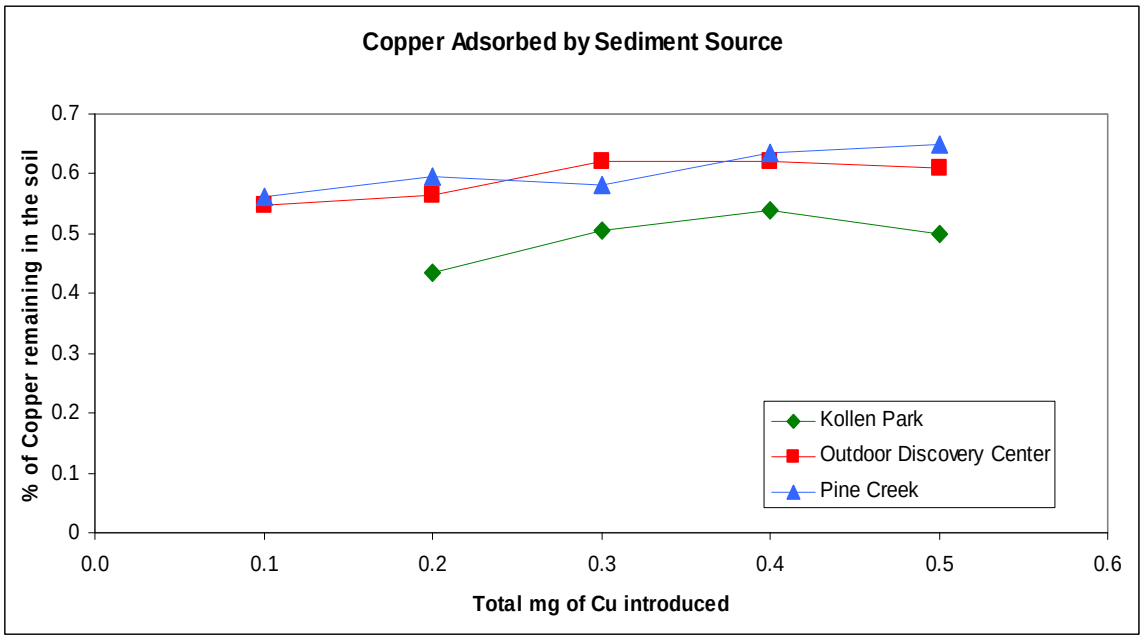


Figure 2. Comparison of amount of copper sorbed according to sample location

Other observations of note are the obvious color change in the *Daphnia magna*. It appears to be a side effect of the copper because the intensity of the color change increased as the concentration of the wash increased. The pre-run *Daphnia* were transparent and very white in color. The post-run *Daphnia* were very dark in color and had small red splotches throughout their bodies.

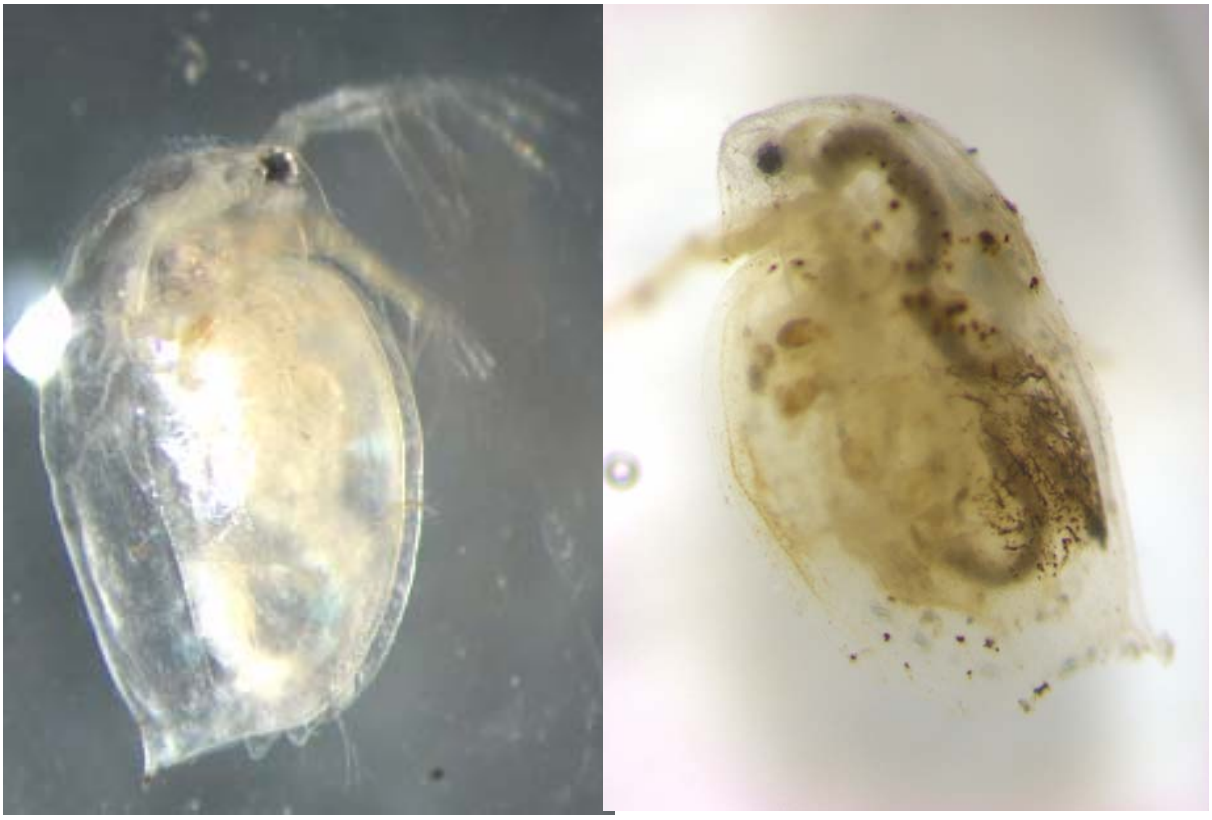

Figure 3. The picture on the left is a *Daphnia magna* pre run while the one on the left is another *Daphnia magna* post run. Note the obvious discolorations and red spots on the post-run *Daphnia*.

## CONCLUSIONS

Copper does have some effect on the survival rate of Daphnia magna, but the soils are not as toxic as we had hoped. It would require either increasing the copper concentration used or the time allotted for the concentration to settle on the soil or removing the rinsing process. A test was done to have statistical data that proves this in which a copper sulfate solution of 10,000ppm was used in which all of the *daphnia* magna were found dead 24 hours later. Another experiment that would potentially work better would be to simply skip the 15 mL wash and complete the addition of the Daphnia right after decanting the copper solution.

The error in the number of Daphnia is accounted for because we count both the number of living and the number of dead which accounts for all the organisms and reduces the risk of inflating the ratio of the number survived to the number deceased. Errors in the concentration are reduced by using serial dilutions so that each dilution will be 10X smaller than the previous dilution whether that which keeps the ratios between concentrations equal. A potential for inaccurate results could be due to the daphnia and their initial health going to the experiment. Daphnia used were not all at the same level of health, some could have been older, or unhealthy in which they might have died for natural reasons and not as a result of the contaminants.

From results of the G-test show Kollen Park to have a value of 26.905 and Outdoor Discovery Center to be 29.058 which are above the critical value of 14.07. These values prove that copper toxicity has significant effects in Kollen Park and Outdoor Discovery Center samples, and that survivorship is dependant on concentration. Pine Creek at 9.355, below the critical value does not have statistical significance.

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