# A Study on the Inhibition and Lysing Threshold for Algal Blooms

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## Abstract:

The aim of this study was to determine dosages of copper algaecide product that would achieve inhibition of algae without cell lysing. Cell lysing is a concern because it could release algal toxins or natural organic matter, a precursor to disinfection byproducts. Algae (cyanobacteria – Anabaena and green algae Chlorella) were cultured under grow lights in alga-gro product. Then they were prepared in 24-well plates while in log-growth phase with copper toxicity dosing to determine the threshold between inhibition and cell-lysis. For cyanobacteria, filament counts did not correlate with copper dose. But length and number of branch filaments diminished even with the lowest dose of copper 50 ppb in 72 hour incubation. Much higher doses caused Chlorella inhibition. Cell lysis did not occur until 50 ppm for cyanobacteria. Chlorella did not appear to be lysed at the highest dose 50 ppm, but were completely inhibited. The concern that water operators have that dosing with copper may cause cell lysis at the recommended dosing for algae control is unfounded and confirms what some recent authors have reported. Furthermore, high levels of copper (20ppm) did not increase disinfection by-product formation in batch experiments, however added humic substances overrode the formation of disinfection byproducts.


## Introduction:


Satellite image of algal blooms in Lake Erie (noaa.gov)

In 2014, an algal bloom in Lake Erie caused a ban on tap water in Toledo for a few days, where nearly half a million citizens could not use the water for bathing, cooking, or drinking. Scientists say that toxin-releasing algal blooms like this are becoming increasingly prevalent in freshwater around the world. In this specific case, Microcystis was the algae that released toxins in the Erie water. There are many possible causes, like climate change warming up water, which is conducive for rapid algae growth, similarly septic tank leakages and farm run-offs release nitrogen and phosphorous , the nutrients for the algae, into the water. However, it is clear that the toxins released from the blooms need to be addressed, regardless of the cause, because these toxins can cause neurological problems such as paralysis and seizures. The presence of toxins also kills nearby wildlife, making this both a crisis for the environment and mankind. Additionally, when these blooms naturally die, bacteria feast on the decaying matter, a process which consumes almost all the oxygen out of the water, thus disrupting the ecosystem in the water, and causing the massive death of fish.

Algal blooms are a common problem in the Great Lakes area. They appear every day, clogging up lakes and rivers. In addition, algal blooms are capable of releasing many harmful toxins into the environment, which can cause various diseases (Ho and Michalak, 2015). The purpose of this experiment is to determine if an inhibitory dose of copper will result in cell lysis, which releases toxins. The second purpose of this experiment is to determine if the copper treatment releases natural organic matter (NOM) into water supply, increasing disinfection byproducts (DBPs). NOM is a major precursor to DBPs such as Trihalomethane. Trihalomethanes (THMs): chloroform, bromoform, and iodoform are regulated in drinking water. The Maximum Contaminant Level for drinking water is 0.08 mg/L or 80 µg/L. This level should not be exceeded in finished water in

order to protect human health because THMs are carcinogenic. Chlorine, however, has been used safely for over 100 years for disinfecting water. Other disinfectants such as UV and Ozone will also create DBPs, such as aldehydes. Chlorine dioxide (a gas) is now used for primary disinfection because it does not form THMs. However, chlorine added to water before distribution in order to maintain free chlorine residual will form THMs (Reckhow & Singer, 1990). Water operators have expressed concern over algal cell lysis upon treatment with copper algaecides, possibly increasing THM formation (El-Dib and Ali 1994). Thus, to solve the toxin problem, we needed to find the level of copper dosage that would not cause lysis, but would still inhibit the toxin-producing blooms.

We selected two types of algae to study in this experiment, one is a cyanobacteria (Anabaena) and one is a green algae (chlorella). There are many different types of algae, but we chose two freshwater types because we live in the Great Lakes area.

Some of the wide-ranging problems with algae are: (1) toxins produced by certain algae/cyanobacteria (2) taste-and-odor compounds – Methylisoborneol and geosmin; (3) organic matter that, when the water is disinfected with chlorine, will produce chloroform, which is a carcinogen. Also, algae cause dead zones because they consume oxygen at night, the algae do produce oxygen during daylight hours. When these massive algal blooms die, bacteria will consume the dead algae and via respiration process, consume large amounts of dissolved oxygen which further depletes oxygen in the water.

While we were experimenting, a publication came out in literature (Kuo-Pei Tsai 2015), "Effects of two copper compounds on Microcystis aeruginosa cell density, membrane integrity, and microcystin release". The study found that mycrocystis toxin was not released at the recommended inhibitory copper dose used in algae control. It takes a much higher dose to release the toxin. Even at a high copper dose with toxin release, levels of toxin in water were lower than when no copper treatment was applied, as algae continue growing in transit time of treatment process.

# Procedure

## Algae Cultures

Algae (cyanobacteria – Anabaena and green algae Chlorella) were obtained from Carolina Biological and cultured under grow lights in 'alga-gro' product (diluted 1:1 with water) as per company instructions. Sub cultures were weekly prepared from the stock cultures. When sub cultures reached log growth phase (exponential population growth usually 3 days after starting a new subculture from an original stock culture) they were ready for copper toxicity testing (this is then referred to as algal inoculums). Copper in our study was in the form of copper sulfate pentahydrate (Aquadrop) with a proprietary organic ligand which is not a chelating agent.

## Copper testing

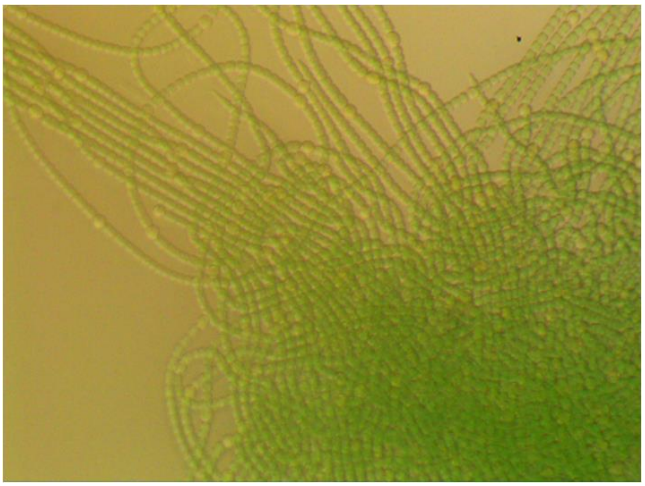
24-Well plates were used for copper toxicity testing. One of the middle rows contained the control, which had no copper treatment added; this had growth media and distilled water added instead of copper solution. Other rows in the plate had variable amounts of copper added. All wells contained the same volume of growth media and either distilled water or copper solution; total volumes were the same. 100 microliters of *algae inoculum* were pipetted into each of the well plates with alga-gro product (diluted 1:1 with water) with different concentrations of copper. After 48-72 hours, algae cells were counted with a hemocytometer under a microscope. Initial cell counts were on the order of log 4.

## Disinfection by-product testing

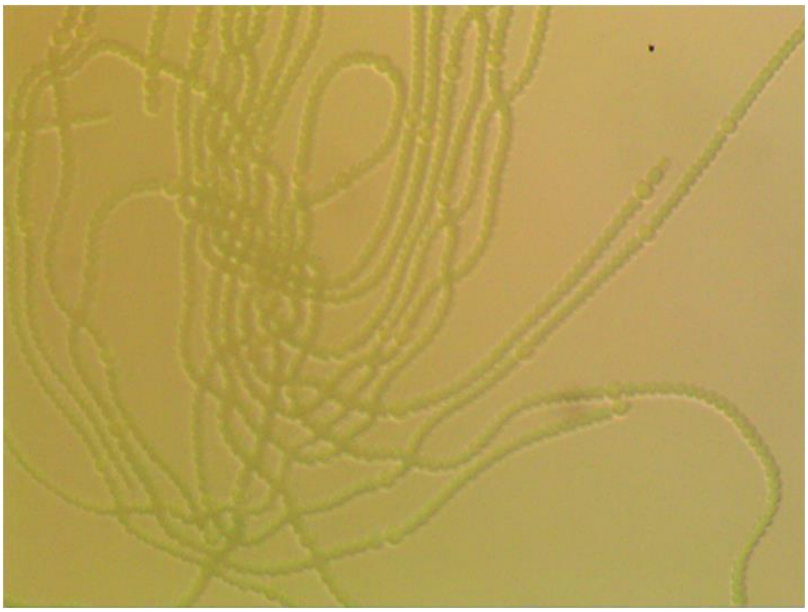
Parameters such as temperature, pH and alkalinity and treatment with copper algaecide (Aquadrop, Ann Arbor) were evaluated to see if they affect disinfection byproduct formation. Algae stock solutions of anabaena were diluted to 500 mg batches. Alkalinity was controlled with calcium carbonate, temperature was adjusted by heated gently with hot plates or cooled in a freezer. pH was adjusted with HCL

or NaOH. A solution of sodium hypochlorite was used to chlorinate the samples as a mock disinfection (3, 6, 9 mg/L doses chlorine tested). Parameters such as temperature (5, 25, and 45 degrees Celsius, pH (5, 7, or 9), and alkalinity (0, 60, 120 mg calcium carbonate added) were adjusted just prior to chlorination. Copper product was diluted and to the algae batches at 0, 4 and 8 ppm (graph values are volumes added where 10 ml of solution refers to 4 ppm and 20 ml refers to 8 ppm final concentration). Water was extracted with MTBE (methyl-tert-butyl ether), as described in EPA Method 551, copper treatment, in a long-term perspective, is the better and then injected into a Shimadzu Gas Chromatograph with electron capture detectors and 30 m x 0.25mm DB-5 and DB-1701 columns to quantify chloroform (THM). A commercial Fossil Fuel was used to adjust the humic acid level used as a variable in the experiment is a better choice than letting the blooms flourish.

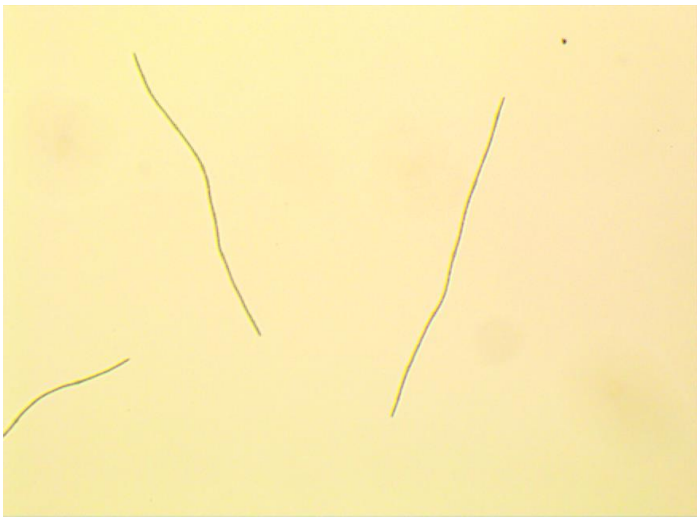
## Graphs/Pictures



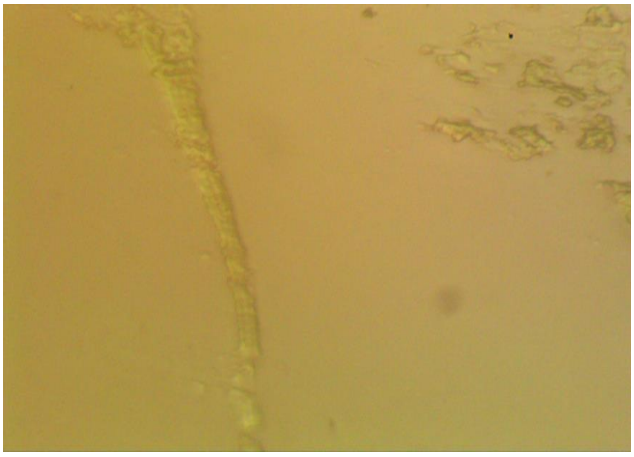
Anabaena in log growth, before treatment:



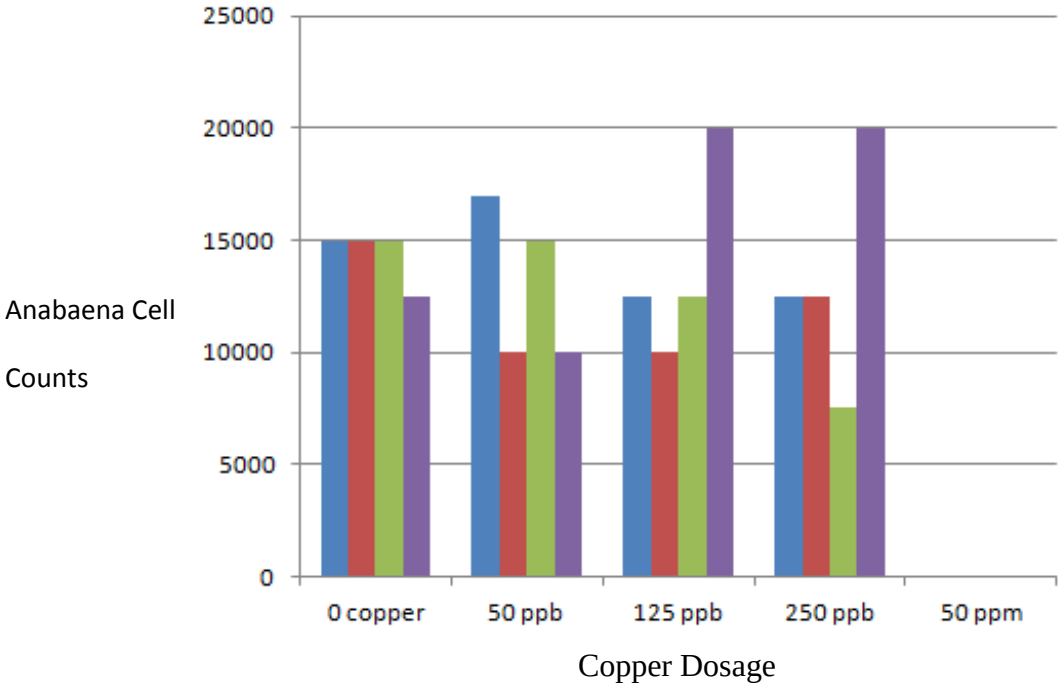
Here is what the Anabaena looked like with the control:



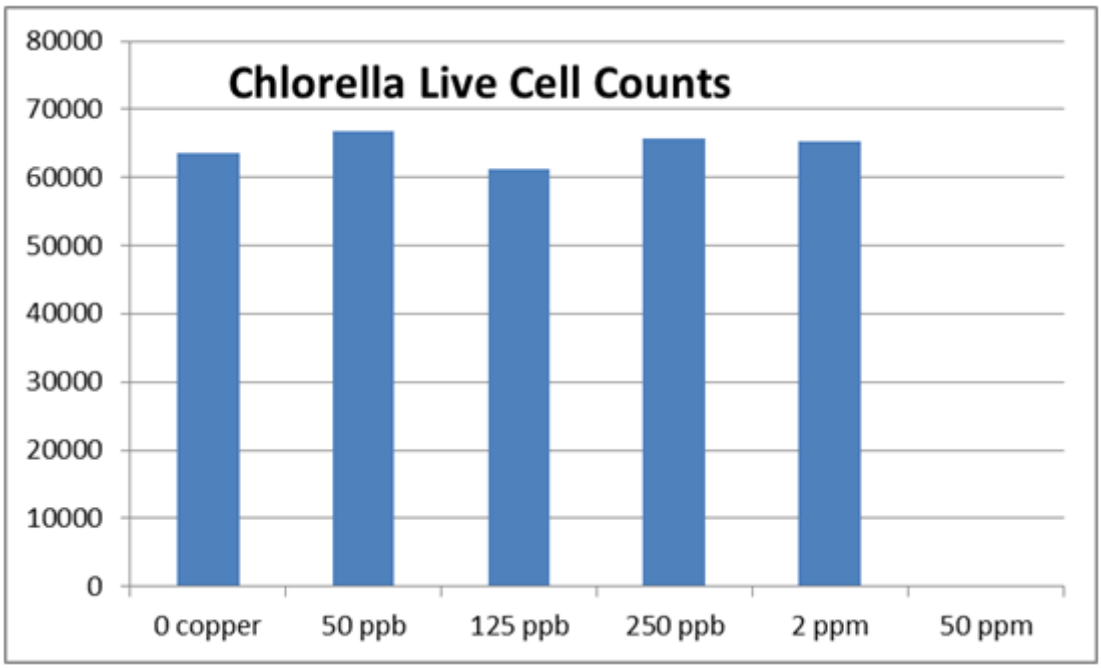
Anabaena after the 50 ppb Copper Treatment



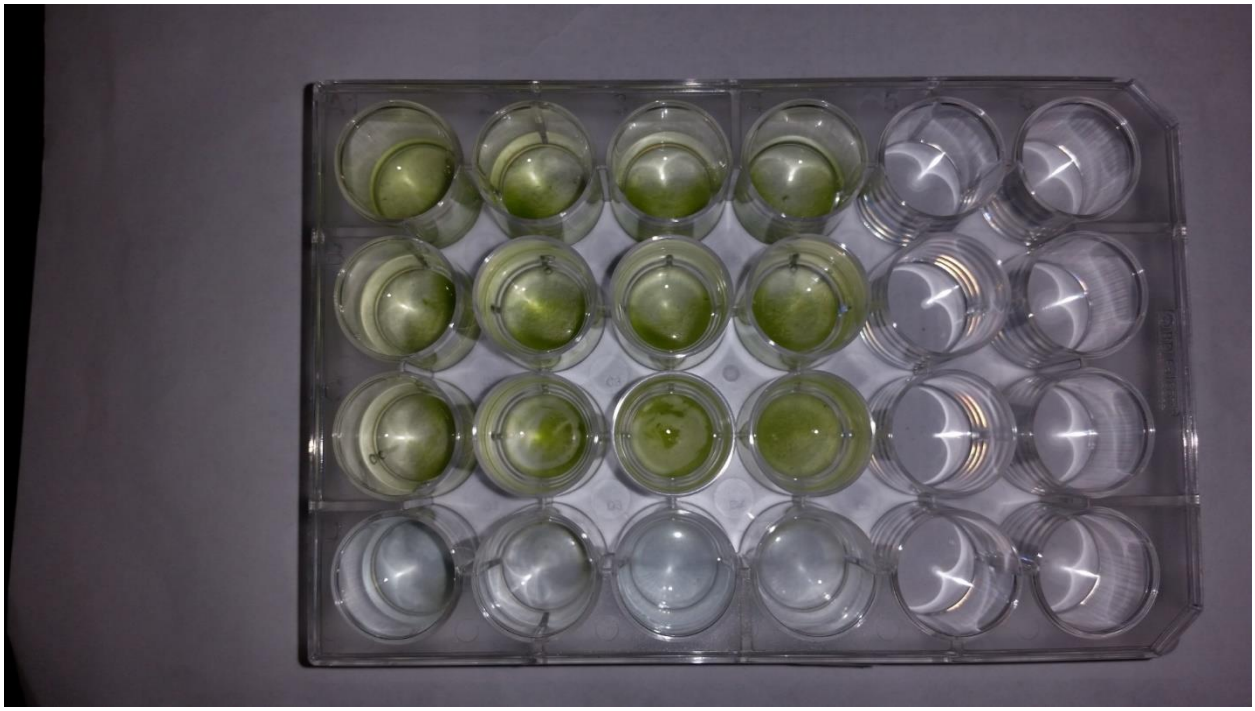
Cell lysis after 50 ppm treatment (Anabaena)




Graph of the living filament counts after 72 hours after the copper treatment (for individual wells): Each color represents different groups of collected data (replicates)




Graph of Chlorella cell count vs. Dosage of Copper (1 plate only – each bar is the average of counts for 4 wells on 1 plate (on 1 plate))



Picture of a well plate containing chlorella

Top row- low copper dose 50 ppb

2nd row- medium copper 125 ppb

3rd Row- Control 0 ppb

Bottom row- high dose 50 ppm

(2 ppm is not shown)

## Results: Chlorella

Chlorella counts were averaged among 4 wells per dose because there was a lot of variability, however the averages showed no difference to the control except for the highest dose of treatment (50 ppm copper) as seen in graph. A photograph of the well plate below shows the four wells at 4 different doses of copper (125, 250 ppb, 2 ppm, 50 ppm). The 50 ppm dose is the bottom row, which appears clear and colorless. Visualized under the microscope the cells can be seen mostly intact, but since there is no photosynthesis, there is no green pigment observed.

With the Chlorella counts, we found some more reliable data that further proved the previously stated conclusion that, with smaller doses up to 2 ppm, there was not much inhibition or cell lysis, but with the large dosage of 50 ppm, there was full inhibition and some lysis. The dose of copper recommended for algae control is generally in the ppb range

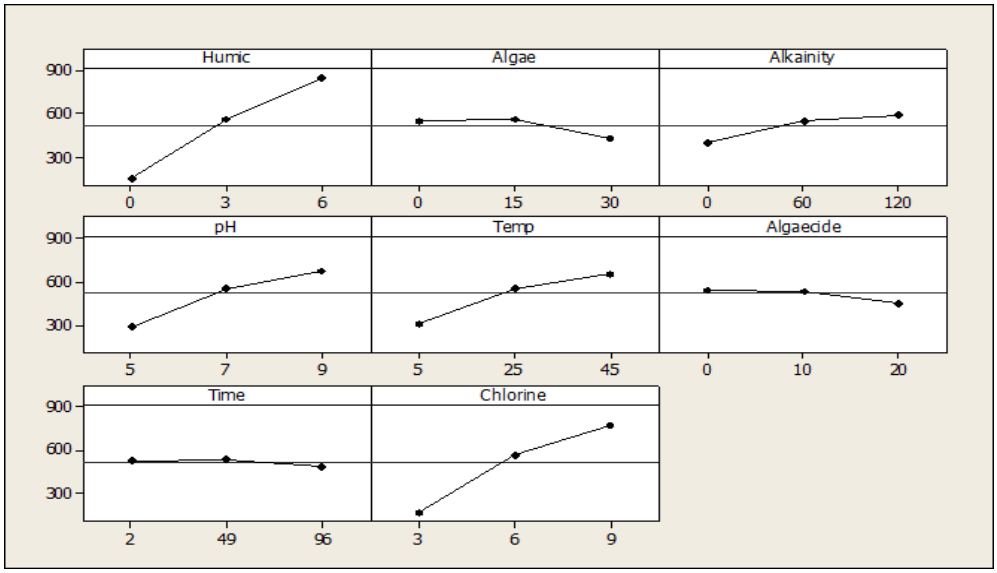
## Results: Anabaena

### Copper Inhibition and Cell lysis:

Algae tested in 24-well plates were dosed with different levels of copper to determine threshold copper inhibition levels. Also, high doses of copper were tested to see if algae cells lysed. For cyanobacteria, anabaena filaments were counted and copper treated batches were not lower than controls (no copper treatment), but were found to be less branched and had fewer cells per filament showing inhibition, as seen in photos 1-3. Individual cells within the filament were not counted, but were observed qualitatively by microscopy. Filament counts were not linear with dose, but smaller when inhibited by copper at doses 50-250 ppb (72 hour incubation), as seen in graph 1. Initial Cell counts were calculated on the inoculate.

But not in the individual wells, where as final cell counts are done in individual wells. It may be possible that inoculate was not homogenous such that when 100 microliter aliquots of inoculate were pipetted into each well, there was not an exact same number of cells per well. Therefore, part of the reason there was not statistical significance in copper inhibition up to 250 ppb may have been a result of this lack of homogeneity in algae counts per well. This made before and after copper treatment comparisons difficult. The reason cells were not counted pre-copper treatment in individual wells is because the cells may be affected by counting. We observed no lysis up to 250 ppb copper. Lysis did occurred at the much higher dose of 50 ppm, photo number 4 shows lysed filament of anabaena.

## Other Information




Graph: main effects plots, y-axis is THM's ppb x-axis variable

## Chloroform DBPs

Another method we used was testing for disinfection byproducts. Chloroform is a disinfection byproduct of the class, trihalomethanes, which is formed when water containing algae and other sources of organic matter is chlorinated. Chloroform was measured in samples of anabaena chlorinated with sodium hypochlorite at 3-9 mg/L.

Batches of diluted algae samples were chlorinated after copper treatment, expect controls had no copper. In addition, some basic water quality parameters of pH, temperature, alkalinity, and organic carbon modified using humic acid were variables studied. Chlorine was added at 3 different doses. A certain amount of time was allowed for the chlorine to react with the organic matter and algae in the water and then the water was extracted with MTBE and analyzed for trihalomethanes. Chloroform was the only major THM formed, so all the THM results are actually chloroform. The amount of chloroform formed was determined as a function of the variables/parameters described.

The graph above shows THM on the y axis and the variable/parameters on the x axis where there are 3 variables per parameter. Most of the parameters were not too significant; the most important was the humic acid added and the amount of chlorine added. This means that the humic acid was the main precursor to chloroform formation. Algae and algaecide did not correlate. Probably not enough algae was used (cultures contained 10^4 cells per liter) compared to humic acid. Therefore, we cannot say with confidence that copper treatment would or would not increase THMs.

## Conclusion

Copper can be used to inhibit algal growth, without worrying about cell lysis, as long as there is no excessive dosage. In both species of freshwater algae, there was no significant lysing with the smaller doses of copper product and there was complete inhibition and lysing with the excessive dosage. However, there must be a threshold between these two ranges, where toxic algal blooms can be inhibited without releasing significant amounts of toxins, and even if some toxins are released, they will never be more dangerous than the amount of toxins released by algal blooms themselves.

## References: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