Effects of Triclosan on Water Quality

Section 34-Team 2-Spring 2019

Aele Bayomi, Ashley Beltre, Danielle Claros, Erica Einhorn

Abstract:

In Lab 3 it was mentioned that many forms of runoff can have great negative effects on water quality index and can affect factors such as temperature, pH, total solids, etc. The hypothesis our team predicted was that the addition of triclosan in an aquatic environment would negatively affect the WQI. The purpose of our experiment was to determine if the addition of triclosan would cause an increase in the pH, DO, Total Solids and CFU. To test our hypothesis, we obtained 6 aquariums and filled each of them with 1 liter of water from Passion Puddle. Three of our control groups remained constant while 1 gram of soap containing triclosan was added to the three experimental groups. After a week, we collected data from the control group and experimental group to test the pH, DO, Total Solids, and CFU. After calculating our p-values, all of our numbers were chance, and we had no significant results. Our results for the experiment refute our hypothesis.

Introduction:

It has been brought to light through numerous articles that triclosan has negative effects on humans, aquatic systems, and the environment as a whole. The compound triclosan, C12H7Cl3O2, is an antibacterial and antifungal agent found in a myriad of household items such as toothpaste, antibacterial soap, detergents, and cleaning solutions (Olaniyan et al, 2016). Triclosan (TCS) and triclocarban (TCC) are among the top 10 most commonly detected organic wastewater compounds for frequency and concentration. TCS has been identified in wastewater

treatment plant (WWTP) effluent at concentrations greater than 10 lg L□1. According to a USGS study monitoring 95 compounds in surface water throughout the United States found TCS to be one of the most frequently detected compounds with surface water concentrations as high as 2.3 lg L□1 (Brasuch & Rand 2010). Based on research performed by Andrea B. Dann and Alice Hontela, triclosan is proven to be extremely toxic to algae, as well as demonstrating harmful developmental and reproductive effects in some fish. Therefore, the potential for further endocrine disruption and antibiotic cross-resistance provides evidence to better regulate the use of TCS in order to avoid human harm (Dann & Hontela et al, 2010). Exposure to high doses of triclosan decreased the levels of some thyroid hormones, although the effects of TCS on humans still has not been determined. However, there have been studies looking into the effects of triclosan on the formation of bacteria resistant to antibiotics and the ways this could impact human health. Overall, research into triclosan has shown that the compound can: alter hormone regulation in animals, might contribute to development of antibiotic resistant germs, and might be harmful to the immune system (Steckelberg 2017). In the experiment conducted by team 2, the effects of triclosan on aquatic environments were tested.

The team hypothesis predicts that the addition of triclosan to an aquatic environment will cause an overall negative effect on the WQI. If the addition of triclosan changes the pH levels to be too acidic or too basic, then the organisms will have a difficult time surviving in the environment. If the addition of TCS changes the DO % in the water to a saturation that the organisms are not used to, they will not be able to properly undergo cellular respiration. If the addition of triclosan in the water decreases the amount of total solids present then there is a chance of water toxicity. If triclosan is added to the water, then the bacteria levels will decrease

because of the antimicrobial properties of triclosan. TCS can alter the aquatic ecosystem, leading to damaging effects on the organisms living within the ecosystem as well as the water quality.

Materials and Methods:

Timeline of Events:

Lab # and Activity Description	In Lab	Outside of Lab
Lab 8 (March 28, 2019) ***-Activity 1*:** ***-Activity 2*:** ***-Activity 3*: Review timeline table and discuss team progress**	1. Collect (7) 1 L Nalgene bottles of sample water from Passion Puddle 2. Prepare and label aquariums 3. Review timeline table and discuss team progress	1. Read lab procedures for pH, DO, total solids, and CFU Assay to prepare for Lab 10.
Lab 10 (April 11, 2019) ***-Activity 1*:** ***-Activity 2*:** ***-Activity 3*: Review timeline table and discuss team progress**	1. Measure ph, DO levels, prepare CFU Assay. 2. Prepare total solids. 3. Review timeline table and discuss team progress	1. Analyze and organize pH and DO data into charts. 2. Lab report preparation 3. Review lab procedures for total solids and CFU Assay analysis.
Lab 11 (April 18, 2019) ***-Activity 1*:** ***-Activity 2*:** ***-Activity 3*: Review timeline table and discuss team progress**	1. Collect total solids data and analyze CFU plates. 2. Discuss data with respect to the hypothesis, lab report, and oral presentation preparation. 3. Review the timeline table and discuss team progress.	1. Analyze and add total solids and CFU data to the lab report. 2. Oral presentation preparation

Materials:

- Triclosan (Dial Hand Soap)
- LabQuest 2
- pH probe
- (6) 1.5 L Containers
- DO probe
- Kimwipes
- Fine tip sharpie
- Squeeze bottle of distilled water
- (6) 150 ml beakers
- Beaker tongs
- White tray
- Designated Oven set to 100℃
- Pan Balance
- 100 ml graduated cylinder
- 6 Serological pipets
- Pipet bulb
- 3 Agar Plates
- P-1000
- P-20
- Blue Tips
- Yellow Tips
- 36 Microfuge tubes

Methods:

For this experiment, 6 liters of water were collected from a lentic source, specifically, the same location in passion puddle. This allowed the control variables to be the temperature of the water and the light source. The independent variable was the amount of triclosan added to the samples of the water. The control group was plain water from passion puddle, while the experimental group was the pond water with the TCS containing soap. The group predicted that the addition of triclosan would negatively impact the water quality. In order to prove this, six 1.4 L aquariums were filled with 1 L of lentic water each. The first three aquariums were the control group, while the other three were the experimental group of water and the addition of soap that contained triclosan, a chemical formula of C12H7Cl3O2. All experimental groups contained 1 gram of dial soap (a TCS containing soap). Based on this set up, both groups were compared

based on the differences in pH, DO, total solids, and CFUs. The results were evaluated using t-tests, the means, and standard deviations. By using these tests, the group was able to distinguish whether triclosan had a significant effect on water quality, or if the results were due to chance.

To measure pH the team plugged in the pH probe into Channel 1 of the LabQuest. Then the tip of the sensor was rinsed with distilled water, which removed any excess water with a Kimwipe. The tip of the probe was then placed into the water sample, starting with Control 1, and allowed to adjust to the new environment for 15 seconds. Data was collected for 10 seconds and then analyzed using the LabQuest which allowed the team to collect the average pH. The process was repeated for all 6 samples of water.

To measure total solids, three 150 ml beakers were obtained, cleaned, and then placed to dry in a drying oven set for 100℃ for 30 minutes. Once the beakers were done drying, they were labeled and measured on a pan balance to obtain the initial weight of each beaker. Before measuring the sample water, the aquarium holding the sample water was swirled in an attempt to disperse any sediments that settled on the bottom of the tank. 100 ml of sample water was then measured using a graduated cylinder and a serological pipette and bulb. After 100ml was measured for all 6 water samples, the beakers were then placed back into the oven and left for a week. After one week, the beakers were removed from the oven and allowed to cool. The total beakers were then measured in grams using a pan balance, in order to determine the difference between the initial weight and final weight, which allowed the team to calculate the total solids. The difference was calculated by subtracting the mass of the empty beaker from the mass of the beaker with the solids. After the data was collected, the beakers were rinsed and the labeling was removed. The beakers were then left to dry.

To start the measurement of the Dissolved Oxygen Saturation, the DO probe was plugged into Channel 1 of the LabQuest. The tip of the sensor was then carefully rinsed with distilled water, and then any excess water was removed with a Kimwipe. The switch on the DO probe was then switched to mg/L. The tip was placed in the sample. The tip did not hit the bottom of the aquarium, but the team did make sure that the metal contacts were submerged. The probe was allowed to adjust to the new environment for 40 seconds. The data was then collected for 10 seconds, and the average concentration was determined by analyzing the data using the Analyze function on the LabQuest. While the probe was still in the sample, the switch was flipped to % and then data was collected for 10 seconds. The average % saturation was determined by analyzing the data using the Analyze function on the LabQuest. This process was then repeated for all 6 samples of water.

To prepare the bacterial CFU, 6 microfuges were shook out and labeled from 0-5. The volume to pipet from a 3x serial dilution in a total volume of 900 ul was calculated . 600ul of sterile water was dispensed into each microfuge tube except 0. The nalgene bottle was swirled briefly before 900 ul of water from passion puddle was pipetted into microfuge tube 0 and vortexed. Then, 300ul of water from microfuge 0 was transferred to microfuge 1 and then vortexed. This was done to the rest of the tubes until each of them had a total volume of 900 ul. Three agar plates were labeled for control 1-3 and experimental 1-2. Each tube was vortexed briefly before spot plating. For each agra gel we spot plated 10ul of the dilution from tube 5 and worked backwards to tube 0. After each spot was plated, the agar gel was left to dry briefly before being turned bottom side up.

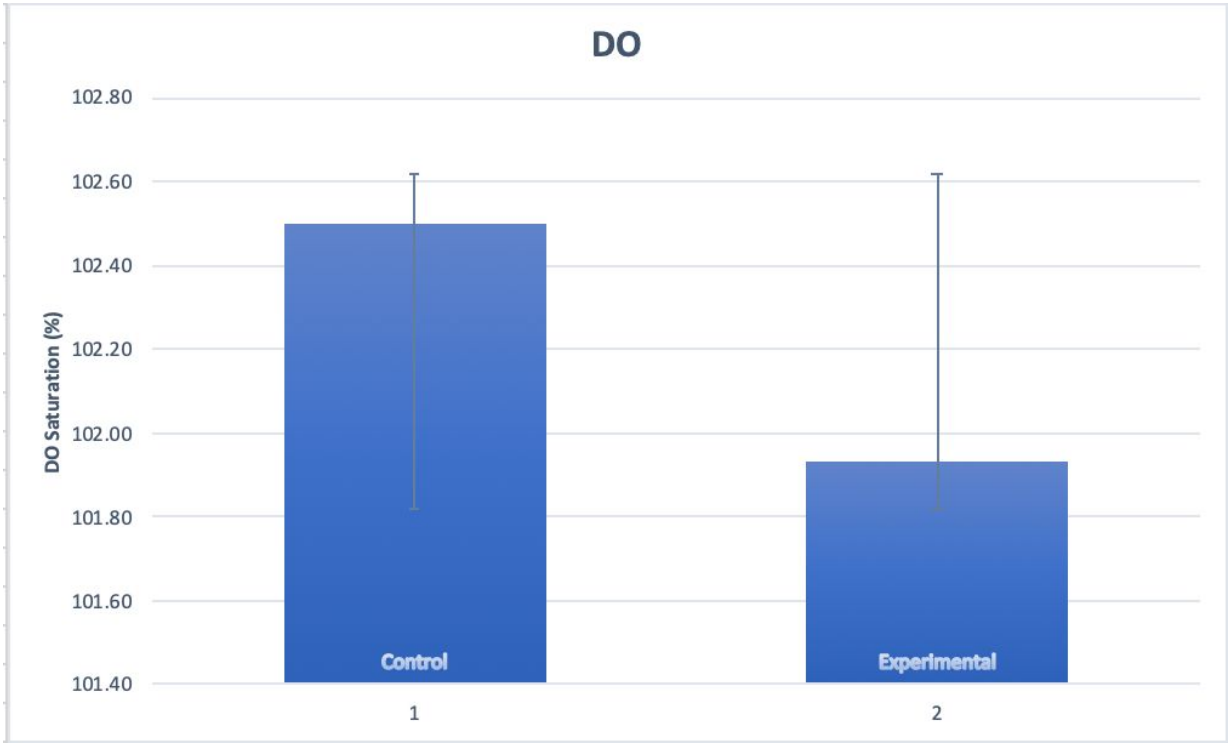
Results:

pH Levels			
Control 1	6.43	Experimental 1	6.27
Control 2	6.51	Experimental 2	6.35
Control 3	6.76	Experimental 3	6.31

The mean pH levels +/- 1 SD for the Control and Experimental group samples. N = 3 for each sample. pH levels were measured during Lab 10, the second week of the Capstone Project. The difference between the Control and Experimental groups was statistically insignificant and due to chance. (p-value = 0.1)

DO Saturation (%)			
Control 1	102.9%	Experimental 1	101.7%

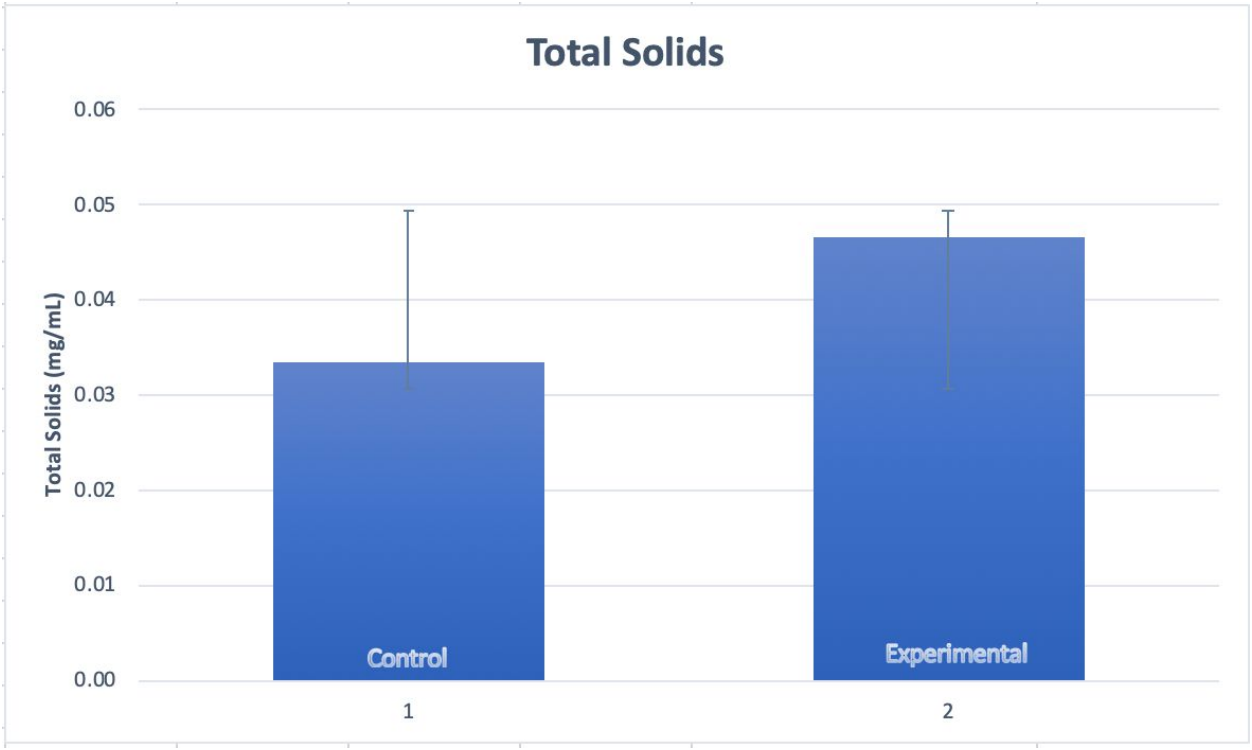
Control 2	103.3%	Experimental 2	98.9%
Control 3	101.3%	Experimental 3	105.2%



The mean DO Saturation Percentages +/- 1 SD for the Control and Experimental group samples. N = 3 for each sample. DO Saturation Percentages were measured during Lab 10, the second week of the Capstone Project. The difference between the Control and Experimental groups was statistically insignificant and due to chance. (p-value = 0.7)

Total Solids	Mass of Beakers (g)	After (g)	Difference (g)
Experimental 1	66.186 g	66.242 g	.056 g
Experimental 2	66.204 g	66.256 g	.052 g
Experimental 3	66.304 g	66.337 g	.033 g
Control 1	65.574 g	65.624 g	.05 g
Control 2	66.891 g	66.922 g	.031 g

Control 3	65.538 g	65.559 g	.021 g



The mean Total Solids +/- 1 SD for the Control and Experimental group samples. N = 3 for each sample. Total Solids was measured during Lab 11, the third week of the Capstone Project. The difference between the Control and Experimental groups was statistically insignificant and due to chance. (p-value = 0.3)

The CFU test was not completed properly, therefore the results are not available for analysis.

Discussion:

If the addition of triclosan changes the pH levels to be too acidic or too basic, then the organisms will have a difficult time surviving in the environment. The average pH for the control

groups was pH 6.44 and for the experimental group it was pH 6.31. Also, based on the p-value of .1, the changes in pH observed in the experiment were insignificant. If the addition of Triclosan changes the DO in the water to a saturation that the organisms are not used to, the organisms will not be able to properly undergo cellular respiration. The average DO for the control group was 102.5% and for the experimental group it was 101.9%. Based on the p-value of .7, the difference was insignificant and due to chance. If triclosan is added to the water, then the total solids levels will decrease which may be a result of water toxicity. The average mg/mL of total solids of the control group was 0.03 mg/mL and 0.05 mg/mL for the experimental group. Based on the p-value of 0.3, the results were due to chance. If triclosan is added to the water, then the bacteria levels will decrease because of the antimicrobial properties of triclosan. However, when completing the test, the agar plates were immediately flipped upside down, causing the water to spread across the plate, mixing the control and experimental group. Therefore, the CFU test will not be included in the data analysis.

The experimental results gathered from this experiment do not match up with the studies reviewed earlier. According to the experimental results of Dann and Hontela, TCS is highly toxic to algae and shows harmful reproductive and developmental effects in some aquatic organisms. While aquatic organisms were not a part of the experiment conducted by team 2, there were no indicators in a change in water quality between the experimental group and control group, so it is unlikely that such a small amount of triclosan would have highly negative effects on algae or other aquatic organisms. The study done by Steckelberg which discussed triclosan and the rise of bacteria resistant to antibiotics cannot necessarily be discussed in relation to the experimental results gathered by team 2 because of the failure to properly complete the bacterial CFU assay.

No data was analyzed based on the bacteria levels in the water. Although, if TCS caused bacteria to become resistant to antibiotics, it would not occur immediately. These results would be from an ongoing experiment because this resistance would be an evolved trait, not an inherited trait.

The hypothesis that the addition of triclosan to the aquatic environment will cause detrimental effects on water quality was not supported based on the test results. All of the experimental results were insignificant, meaning 1 gram of triclosan per liter of water had no legitimate effect on water quality. Although, these results do not mean that triclosan has no effect on water quality. The experiment was set up in a way meant to mimic runoff that would alter an aquatic ecosystem. The amount of triclosan in runoff would not make-up a large percentage of the contents of pond, so 1 gram of triclosan was the chosen value to be tested. However, it was not taken into account that runoff is continuous, so ideally, a greater amount of triclosan should have been added to the experimental group to more accurately test the effects of water polluted with triclosan.

Works Cited

Brausch, J. M., & Rand, G. M. (n.d.). A review of personal care products in the aquatic environment: Environmental concentrations and toxicity. *El Sevier,* 1518-1532. Retrieved February 27, 2019.

[BRL117] Biological Research Laboratory 01:119:117 Laboratory Manual: Aquatic Ecology and DNA Barcoding, Spring 2019. Rutgers University, New Brunswick, NJ.

Dann, A.B, & Hontela, A. (2010). Triclosan: environmental exposure, toxicity and mechanisms of action. *Journal of Applied Toxicology*. [Internet]. [cited 2019 Feb];31(4):285–311. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/jat.1660

Xin X, Huang G, An C, Raina-Fulton R, Weger H. 2019. Insights into Long-Term Toxicity of Triclosan to Freshwater Green Algae in Lake Erie. Environ. Sci. Technol. 53(4): 2189-2198

Available from: https://pubs.acs.org/doi/10.1021/acs.est.9b00259

Olaniyan, L. W., Mkwetshana, N., & Okoh, A. I. (2016). Triclosan in water, implications for human and environmental health. *SpringerPlus*, *5*(1), 1639. doi:10.1186/s40064-016-3287-x