The Effects of Temperature on Leaching of Antimony from Plastic Bottles and Subsequent Impact on Brine Shrimp Hatch Rate

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Abstract

For the past 120 years, Bisphenol-A (BPA) has been used in plastic products, such as water bottles, due to its strength. BPA, which contaminates 93% of the human population (Houlihan, et. al, 2011), has started to be replaced by other materials that leach antimony, a harmful contaminant commonly found in polyethylene terephthalate (PET) water bottles. Additionally, as global and ocean temperature start to rise, antimony leaching levels have been predicted to increase as studies by Fan, et. al and Westerhoff, et. al observed a directly proportional relationship between water temperature and the leaching of antimony. This investigation examined the effect of varying saltwater temperatures on the leaching of antimony from PET plastic water bottles, and the subsequent impacts on brine shrimp hatch rates. Six buckets of saltwater were heated to appropriate temperatures (two of each temperature - 17 degree C, 22.5 degree C, and 28.1 degree C - one control and one experimental). Antimony, ammonia, pH, phosphate, and nitrate levels were measured over the three weeks. Then, brine shrimp hatching began, using the experimental and control water. The brine shrimp were allowed to hatch for 48 hours, after which a net was used to separate and count the hatched eggs. The results showed an increase in antimony in the plastic bottle groups; however, the measurement was not quantifiable. Additionally, results showed an increase in phosphate, ammonia, and pH levels for buckets with the water bottles. This investigation has shown that as the temperature of water increases, antimony levels increase and brine shrimp hatch rates decrease.

The Effects of Temperature on Leaching of Antimony from Plastic Bottles and Subsequent Impact on Brine Shrimp Hatch Rate

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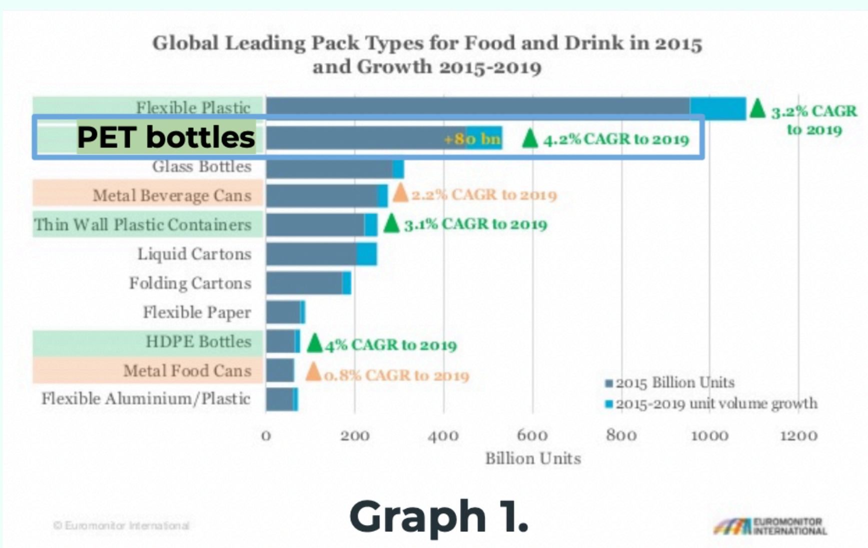
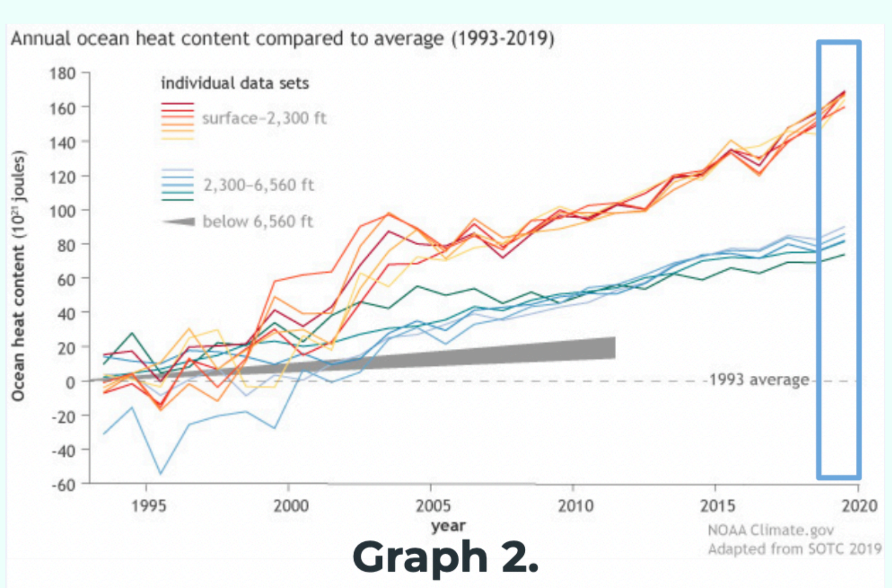
Background Research

Studies conducted over the past 20 years show that BPA contaminates 93% of the human population, with effects including health effects on the brain, prostate gland of fetuses, infants, and children, children's behavior, and increased blood pressure (Bauer). States soon demanded BPA to be phased out in formula and baby bottles. Today, plastic bottles do not contain BPA; however, this toxic chemical has been replaced with other materials that leach antimony, a regulated contaminant that poses both acute and chronic health effects such as eyes, skin, lung, heart, and stomach problems (CDC).

Polyethylene terephthalate (PET) is used for its transparency, light weight, and CO2 impermeability. Additionally, although PET is BPA free, it is also associated with many of the same health risks, such as stunted growth, reproduction issues, low energy levels, body balance issues and inability to process stress. About 15 years ago, the presence of antimony in water in PET water bottles was found. Scientists have reported significant concentrations of antimony in bottled waters and linked them to the use of antimony in the production of PET bottles. Antimony trioxide is a common catalyst in the production of PET resin and antimony remains in the objects produced.



Graph 1. Global PET bottle production continues to grow



Graph 2. Ocean temperature continues to rise over time

Graph 1 shows the increase in PET bottle use from 2015-2019 and Graph 2 shows the ocean temperature rise from 1995-2020. As ocean temperature and PET bottle production continue to rise, and people keep plastic bottles in cars during the summer, rising temperatures can drastically expedite the leaching process. The purpose of this study was to observe the impacts of varying temperature on the leaching of antimony and its effects on brine shrimp hatch rates. Brine shrimp are model organisms for humans in this study.

This investigation aimed to observe the effects of the increase of temperature (17 degrees Celsius, 22.5 degrees Celsius, and 28.1 degrees Celsius) on the leaching of antimony from Crystal Geyser (a known PET brand of plastic water bottles) water bottles. Additionally, the subsequent impacts on Brine Shrimp hatch rates as model organisms for humans was observed. It was hypothesized that if the temperature increased, the leaching of antimony would increase, decreasing the brine shrimp hatch rates as they are no longer in their optimal environment. Results from this investigation can be used to prove that plastic bottles should not be used because they leach antimony at increasing temperatures, common during the summer season in cars and oceans.

Methods

To begin the experiment:

1. Set up 12-five gallon buckets with heaters for each bucket
2. Fill each bucket with 1.5 gallons of water.
3. Add six ounces of instant ocean mix to each bucket.
4. Allow the buckets to acclimate to the assigned temperatures using the aquarium heaters.
5. Label the buckets: bucket 1: 17 degrees Celsius no plastic, bucket 2: 17 degrees Celsius with 3 bottles of plastic, bucket 3: 22.5 degrees Celsius no plastic, bucket 4: 22.5 degrees Celsius with 3 bottles of plastic, bucket 5: 28.1 degrees Celsius no plastic, bucket 6: 28.1 degrees Celsius with 3 bottles of plastic.
6. After acclimation, measure and record the starting levels of pH, ammonia, phosphate, nitrate, and antimony.
7. Add three bottles of water to the buckets requiring plastic contamination, weighing them down with glass marbles.
8. Measure and record antimony levels until they level off. Set up the video cameras and laptop to record the brine shrimp hatching. Then, prepare the containers for brine shrimp hatching.

To measure the levels of antimony, two methods were used: antimony test strip analysis and FeCl3 analysis.

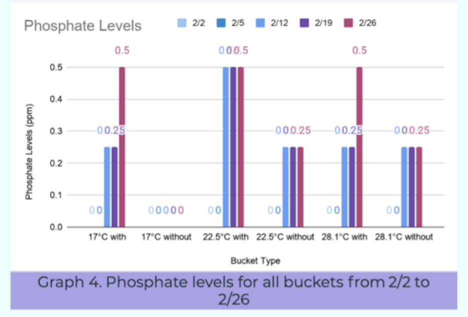
A. **For the test strip analysis:**
   1. Prepare 30 mL of diluted HCl in small beakers.
   2. Using a dropper, add a couple drops of the test solution to each test strip. Then dip the test strip into the HCl for one second.
   3. Watch the test strips for any orange spots which indicate the presence of antimony.
   4. Repeat the steps for each test solution.

A. **For the FeCl3 Analysis:**
   1. Prepare 30 mL of FeCl3 in small beakers.
   2. Using a dropper, add an eyedropper full of the test solution to each beaker.
   3. Using a stirring rod, stir the solution and watch for the formation of any precipitate, which indicates the presence of Antimony. Repeat the steps for each test solution.

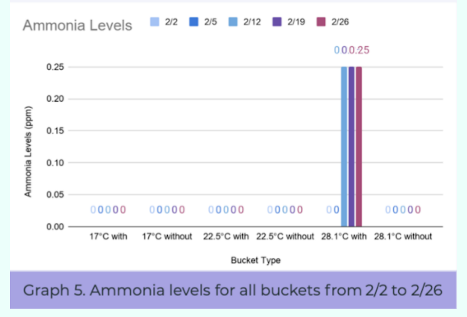
To hatch the brine shrimp:

1. For each plastic bucket, label 4 containers according to the group and bucket from which the water originated.
2. Place a white paper towel under the container to ensure the eggs are visible.
3. Transfer 2 liters of water from each bucket to the small container, moving the heater with it. Count out 50 brine shrimp eggs and evenly distribute them on the bottom of the hatching container.
4. Position the camera above the container. Set a timer for 28 hours. At the end of 48 hours use a small net to remove and count the brine shrimp.
5. At the end of the experiment, transfer the brine shrimp which hatched to a bucket with an airline and feed them. Repeat these steps for each of the groups of buckets.

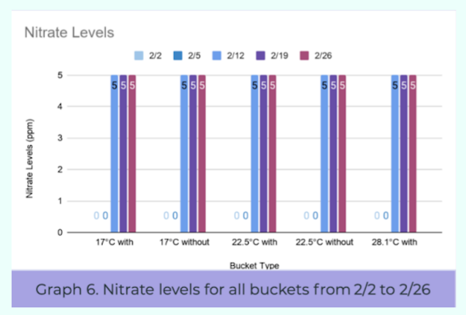
Results and Graphs



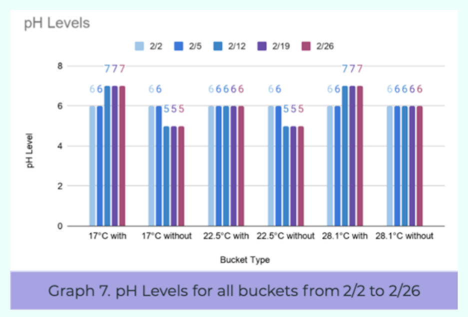
Graph 4. Phosphate levels for all buckets from 2/2 to 2/26



Graph 5. Ammonia levels for all buckets from 2/2 to 2/26



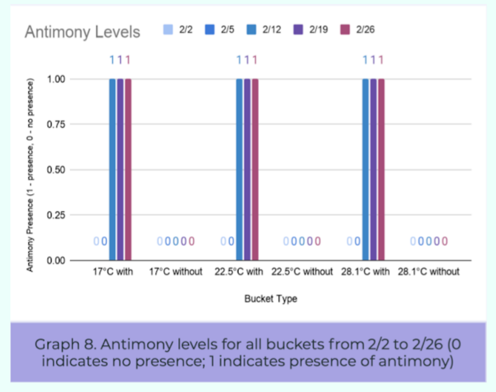
Graph 6. Nitrate levels for all buckets from 2/2 to 2/26



Graph 7. pH Levels for all buckets from 2/2 to 2/26

Graphs 4 - 7

Buckets with plastic bottles experienced a **50% average increase** in phosphate levels, **8% average increase** (only in the 28.1 degrees Celsius with plastic bucket) in ammonia, and an **11% average increase** in pH levels. All buckets experience a 500% average increase in nitrate levels.



Graph 8. Antimony levels for all buckets from 2/2 to 2/26 (0 indicates no presence; 1 indicates presence of antimony)

Image 1. Precipitate from $FeCl_3$ Testing for 28.1°C with plastic

Graph 8 and Image 1

All buckets with plastic experienced an increase in antimony levels as revealed by the $FeCl_3$ test. Because the minimum threshold for the test strips was 0.5 mg/m³ (UK limit), antimony levels were concluded to be below the threshold. However, as shown by the circles in Images 1, 2, and 3, there was precipitate that formed from the FeCl3 indicating the presence of antimony.

Results and Graphs

Image 4. Brine Shrimp hatching in 17°C water without plastic after 48 hours

Image 5. Brine Shrimp hatching in water with 22.5 degree water with plastic after 48 hours.

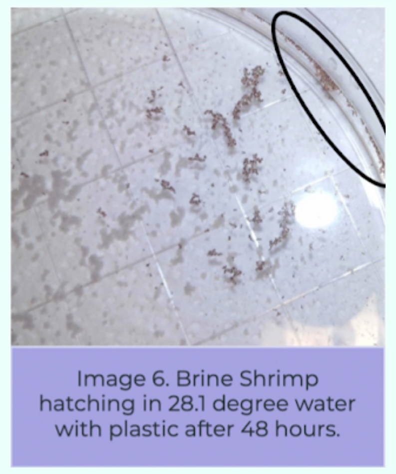


Image 6. Brine Shrimp hatching in 28.1 degree water with plastic after 48 hours.

Images 4 - 6

For brine shrimp results, less brine shrimp hatched in the water exposed to higher temperature (28.1°C) when compared to the eggs in the water without plastic at 17°C. The leaching of antimony did reduce the hatch rate of brine shrimp, signifying that this could reduce the gestation period of humans. Premature babies are more likely to have chronic health issues such as infections, asthma, and feeding problems.

Conclusions

The hypothesis was **supported** as the increase in temperature caused a presence of antimony in buckets with plastic, when compared to no presence of antimony in buckets without plastic. Additionally, less brine shrimp hatched in the water exposed to higher temperatures meaning the leaching of antimony impacts the hatch rates of brine shrimp. In relation to a larger real life application, this study supports that at high temperatures, people are more susceptible to the negative effects of antimony. Microplastics and plastics have played an impactful role in society, but it is important to address and understand the negative effects of plastic on humans, the environment, and other organisms.

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