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Effects of Three Road Ice Fighters on Caddisfly Survival

Abstract

Since salt and other toxins have affected larvae in other studies, we hypothesized that the samples with table salt, MgCl brine, or beet juice brine will have a lower LT50 than the control group. We also hypothesized the larvae would have the highest mortality in salt and the lowest mortality in beet juice brine. The null hypothesis will be that table salt, beet juice brine, and MgCl samples won't differ on the control's group LT50.

Introduction:

Every winter a number of toxins are spread over roads to make driving possible, but this disturbance comes with a price that our ecosystem has to pay. The use of rock salt (NaCl) on U.S. roads has skyrocketed in the last 65 years, and chloride (Cl) concentrations in waters of the northeast have risen as a consequence. The mobility of salt in water leads to its potential problems in the environment. These problems include toxicity to plants and fish, groundwater contamination, and human health interactions, particularly salt intake and hypertension (Jackson, R. B., & Jobbágy, 2005). Due to constant use of salt, Magnesium Chloride (MgCl) and beet juice brine during wintertime that end up in freshwater streams after the ice melting, many non-target aquatic insects are exposed, and in order to evaluate the effects this toxins have in our environment, we decided to test an indicator species for this experiment: The caddisfly. Trichoptera or caddisflies are holometabolous insects with aquatic larvae that, together with the Lepidoptera, comprise the Amphiesmenoptera. (Kjer, K. M., Blahnik, R. J., & Holzenthal, R. W, 2002). Most caddisflies are herbivorous and eat decaying plant tissue and algae that they scratch from rocks. Caddisfly larvae are also known to prefer microhabitats with large, stable substrate and high water flow velocity (Georgian, T., & Thorp, J. H, 1992). Studies on similar larvae have been done by Beadle, L. C., & Shaw, J. (1950). The studies showed that in both *Aedes aegypti* and *Sialis lutaria* larvae the haemolymph (needed for circulation) osmotic pressure rises when the external salt concentration is increased, so that the haemolymph remains hyper-osmotic to the medium. Regulation of the haemolymph salt concentration begins to break down when the external salt concentration is increased to a level roughly equivalent to the normal total concentration of the haemolymph. It is known thanks to this studies that for freshwater insects, external concentrations greater than 30-40 % of sea water are rapidly fatal.

Since salt and other toxins have affected larvae in other studies, we hypothesized that the samples with table salt, MgCl brine, or beet juice brine will have a lower LT50 than the control group. We also hypothesized the larvae would have the highest mortality in salt and the lowest mortality in beet juice brine. The null hypothesis will be that table salt, beet juice brine, and MgCl samples won't differ on the control's group LT50.

Methods:

In order to test the effects of ice fighters on caddisfly survival, we did 100 repetitions in total, with 5 caddisflies each. With the help of three people, 3 different substances were tested: beet juice brine (provided by the Bonner Springs road maintenance), MgCl brine (provided by the Kansas Department of Transportation), and table salt. For each substance we tested 3 different concentrations. The solutions were prepared 5 days before the caddisflies were going to be tested, by adding the toxin to water collected from the stream in which we found the caddisflies. The samples were kept in the refrigerator until the time of testing. Our concentrations were of 0.25%, 0.5%, and 1% for each road treatment: salt, beet juice and MgCl. Finally, our control group was a 0% solution. All the samples were aerated for approximately 30 seconds before being placed on petri dishes, and had a temperature of 10°C. The result was:

Number of repetitions	Toxin	Concentration
10	N/A (control)	0%
10	Salt	0.25%
10	Salt	0.5%
10	Salt	1%
10	MgCl	0.25%
10	MgCl	0.5%
10	MgCl	1%
10	Beet Juice	0.25%
10	Beet Juice	0.5%
10	Beet Juice	1%

We had a total of 100 clear plastic 47 mm petri dishes. Inside of each of them, we placed 5 caddisflies one by one using forceps and as they were added timers were set for each treatment. They were under fluorescent lighting during while being tested. The 500 caddisflies we used were collected one by one using forceps. They were taken from the Mill Creek Streamway in Olathe, the same day we tested them. After one hour of exposure, we checked the movement of each caddisfly, and if the caddisflies were not moving when we checked them, we gently picked them up with forceps and put them back in the water to induce movement. Caddisflies that showed no movement after this were recorded as dead. We checked for mortality hourly for 4 hours in order to determine the LT50 for each concentration.

Results:

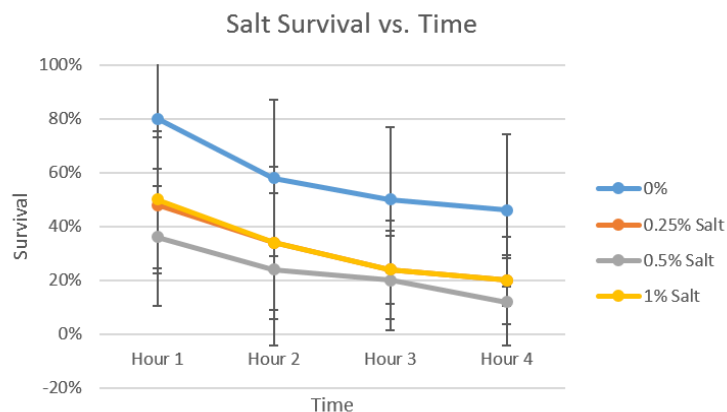


Figure 1. Results of table salt exposure in 3 different concentrations over time compared to a control group.

In the table salt sample, the highest survival rate was shown by the control group and the lowest one was shown by the 0.5% concentration. Finally, the 0.25% and the 1% concentration had very similar results. It's clear that there's a major survival breakdown on the first hour but then the survival decreases more smoothly for the 3 last hours of testing.

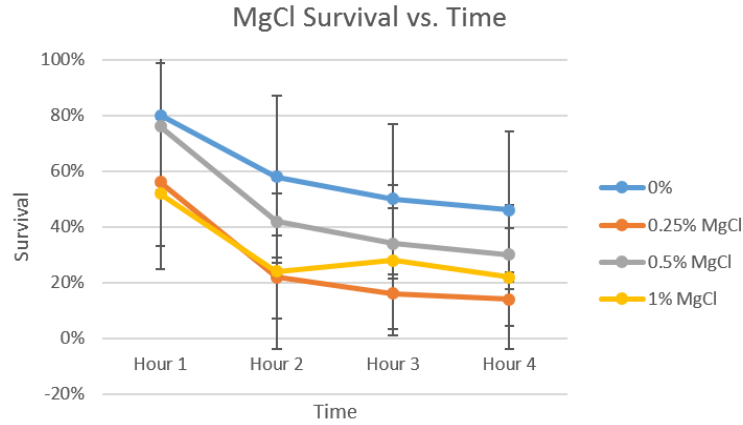


Figure 2. Results of MgCl brine exposure in 3 different concentrations over time compared to a control group.

In the table MgCl sample, the highest survival rate was shown by the control group too, and the lowest one was shown by the 0.25% MgCl concentration. The second highest survival rate was shown by the 0.5% concentration and the 1% MgCl sample had the third highest survival rate. On this test there's also a major survival breakdown on the first hour and then the survival keeps decreasing with time but more smoothly.

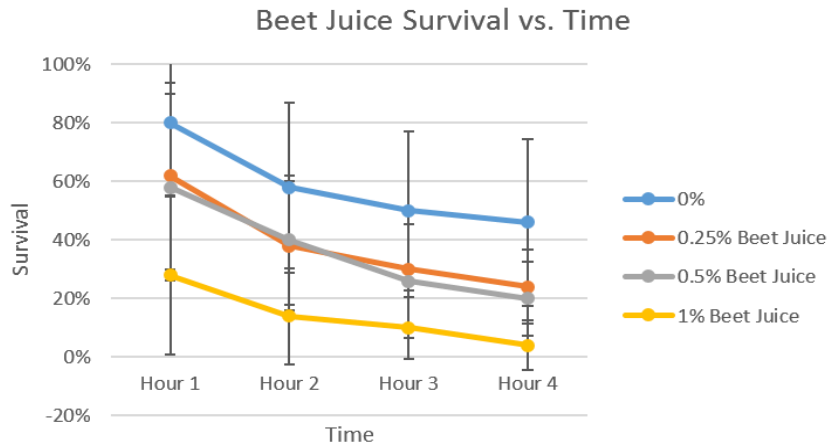


Figure 3. Results of Beet juice brine exposure in 3 different concentrations over time compared to a control group.

The beet juice sample showed the most logical survival order. The survival rate shown from high to low was: control group (0%), 0.25% concentration, 5% concentration, and 1% beet juice concentration. Just like the other two tests, there's a major survival breakdown on the first hour and then the survival rate decreased more smoothly on the last 3 hours of testing.

Treatment	Estimated LT50
Control	3 hours
Salt 0.25%	1 hour
Salt 0.5%	Less than an hour
Salt 1%	1 hour
MgCl Brine 0.25%	1 hour and 15 minutes
MgCl Brine 5%	1 hour and 45 minutes
MgCl Brine 1%	1 hour
Beet Juice Brine 0.25%	1 hour and 30 minutes
Beet Juice Brine 0.5%	1 hour and 30 minutes
Beet Juice Brine 1%	30 minutes

Discussion:

The findings in this experiment supported the initial alternate hypotheses where we stated that the samples with table salt, MgCl brine, or beet juice brine would have a lower LT50 than the control group. We were pretty confident on this one because toxins like salt in high concentrations usually harm insects, and we were expecting a similar reaction for the MgCl and the beet juice brine. We also hypothesized that larvae would have the highest mortality in salt and the lowest mortality in the beet juice brine but the experiment proved us wrong. This was surprising, we thought the beet juice wouldn't be so tough on the larvae since it's a more natural component but we were wrong. When we were performing the experiment the beet juice

samples had a very dark color and a strong smell (similar to chocolate). It was also very thick which could've been what killed the caddisflies. The null hypothesis was that table salt, beet juice brine, and MgCl samples wouldn't differ on the control's group LT50 but this one was rejected as we were expecting. Most toxins do have some type of effect over living things. The beet juice and MgCl brine might contain some amount of salt already which made them more lethal, or there's always the possibility of having unhealthy caddisflies in some solutions just by chance. The last possibility we thought about is that the death of some caddisflies might have caused more death because of all the decomposing matter.

When working on experiments, there are always things that could've been done better to have more precise results. By performing our experiment we learned that we needed more repetitions to minimize the error. Also, having more people counting the caddisflies would be better, to be able to test all of them at the exact same time and avoid undesired variables. We also thought that by using the exact same road salt (used to defrost roads) instead of the table salt we used and also by doing the experiment under a colder temperature, we would imitate the real world environment better, and therefore have more accurate data of what's going on out there.

Our experiment is great to understand changes in our real world environment. Thanks to the data we collected, we can understand better how toxins we use during winter can be destroying the balance of some ecosystems. Also, we learned that beet juice is too harmful for aquatic insects, and that salt is the least harmful. This can be taken into account when choosing ice fighters. In conclusion, we wouldn't recommend using beet juice in high doses or using this ice fighters harmful ice fighters near streams or water ecosystems in general.

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