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Julie Deslauriers
University of Central Florida, deslauriersjulie@knights.ucf.edu

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Preventing Introductions to Sustain Healthy Ecosystems: Establish Eradication Protocols for a Popular Aquarium Seaweed

By: Julie Deslauriers
Faculty Mentor: Dr. Linda Walters
UCF Department of Biology

ABSTRACT: Aquarium release, a vector that introduces non-native species, recently caused the costly invasion of the green macroalga (seaweed) *Caulerpa taxifolia* along the Californian, Mediterranean, and Australian coasts. *C. taxifolia* was classified by the International Union for the Conservation of Nature as one of the world's 100 worst invasive species and cost California over $7 million to control with black tarps and chlorine bleach. Since the incident, educational efforts at conferences and conventions have influenced more than 50% of surveyed hobbyists to switch to another green macroalga, *Chaetomorpha*, as their primary alga in their saltwater tanks. *C. taxifolia* and *Chaetomorpha* have many similarities, including broad environmental tolerances, high nutrient uptake rates, and the ability to survive and reproduce from very small fragments. Previous studies have focused on physical ways to responsibly eliminate unwanted *Chaetomorpha* in home aquariums (e.g. boiling, freezing, etc.), yet the only effective eradication method documented is acetic acid. The goal of this project was to determine the minimum treatment combination (quantity and exposure duration) of acetic acid needed to eradicate aquarium *Chaetomorpha*. We found that a 4% solution exposed for 10 minutes caused 100% mortality within 24 hours. We additionally tested and determined that commercially available vinegar (4-6% acetic acid) was likewise effective, which creates a convenient and safe eradication method accessible to all households.

KEYWORDS: aquarium release, *Chaetomorpha, Caulerpa taxifolia*, non-native species

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INTRODUCTION

The aquarium industry is worth over $1 billion worldwide and is a prominent hobby for millions of individuals (Livengood and Chapman 2014). The import value of ornamental fish is over $278 million, with the United States leading the industry as the single largest importer (FAO 1996-2005, Chapman 2000). Additionally, there are approximately 1,539 species of fish, 102 species of corals, and 293 species of invertebrates involved in the aquarium trade (Livengood and Chapman 2014). Although the industry was once dominated by freshwater organisms, marine organisms have recently become more popular due to their rising popularity in pop culture and advances in marine equipment technology.

Accompanying the rise of the marine industry, "aquarium release" has also increased. Aquarium release is a worldwide concern as a pathway for the introduction of non-native species into new environments. Novel species can be introduced intentionally through direct release of organisms or unintentionally through the dumping of live cells found in used aquarium water (Calado and Chapman 2006). When novel species enter new environments, native species lack evolutionary history and are often unable to form timely defenses. Absence of biotic controls can also lead to the establishment of invasive species and subsequent habitat degradation (Donnelly 2014).

The invasion of *Caulerpa taxifolia* in natural ecosystems is one of the best-documented examples of aquarium release (Walters 2009). This green-feathered macroalga stressed the economy and deteriorated the ecosystems off the coasts of South Australia, the Mediterranean, and California (Meinesz et al. 1996, Relini et al. 1998, Williams and Smith 2007). The degradation of the ecosystem caused by *C. taxifolia* in these locations led the International Union for the Conservation of Nature to place *C. taxifolia* on the world's 100 worst invasive species list (Lowe et al. 2004). In order to cope with this deadly alga, over $7 million was spent in California to eradicate the invasive populations. The primary method used was chlorine bleach placed under secured black tarps covering the *C. taxifolia* beds to kill cells and prevent photosynthesis (Anderson 2007).

Knowledge of the invasion of *Caulerpa taxifolia* led more than 50% of individuals attending a national aquarium hobbyist convention to no longer use *Caulerpa*, but rather instead use *Chaetomorpha* for their tropical or subtropical reef aquaria, making it the most commonly used algae in home saltwater aquariums (Walters et al. 2011). *Chaetomorpha*, referred to as spaghetti algae, is a genus consisting of unbranched filamentous green macroalgae. *Chaetomorpha* has broad environmental tolerances and high nutrient uptake and growth rates (Xu and Lin 2008). Due to the ease of hosting *Chaetomorpha* and its popularity among hobbyists, it is very likely that some cells may end up in the environment via aquarium dumping or accidental disposal into the waterway.

Many comparative studies between *Caulerpa taxifolia* and *Chaetomorpha* have been conducted to assess the safety of using *Chaetomorpha* as the major macroalgae in marine aquariums. *Caulerpa taxifolia* can survive and reproduce from fragments as small as 10mm in length (Smith and Walters 1999). Data shows that *Chaetomorpha*, our genus of interest, can proliferate from a single undamaged cell as small as 0.5mm, suggesting that this macroalgae poses an even greater invasive threat than *Caulerpa*. In warm environments, such as those present in tropical and subtropical regions, 0.5mm fragments of *Chaetomorpha* have a 72% survival, 1mm fragments have a 90% survival, and fragments 2mm or longer also have a 90% survival rate (Odom 2012).

Aquarium release of *Chaetomorpha* may pose an invasive threat even in areas where *Chaetomorpha* species are native, similar to what occurred during the massive biological invasions of *Caulerpa.* Meinesz and Boudouresque (1996) suggest that the strong selection pressures exerted on aquarium *Caulerpa* could give it a survival advantage over native *Caulerpa* species. Locally, the introduction of the aquarium *Chaetomorpha* into the Indian River Lagoon in Florida is a concern due the potential for genetic recombination and speciation during years of isolation in aquarium environments. According to the Smithsonian Marine Station at Fort Pierce (2014), there are five species of the *Chaetomorpha* genus documented as native to the Indian River Lagoon, including *Chaetomorpha aerea, Chaetomorpha brachygona, Chaetomorpha gracilis, Chaetomorpha linum,* and *Chaetomorpha minima*. *Chaetomorpha* suppliers continually pull from a limited gene pool and years of genetic isolation may create a different population that could act as a thriving invasive species in the Indian River Lagoon. Furthermore, Florida is greatly affected by invasive species due to island development, tropical climate, and high seasonal rainfall (Masterson 2007).

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Many scientists have recognized *Chaetomorpha's* threat to native species and have tested effective eradiation measures. In summary, the exposure time and concentration of tested chemicals to cause growth reduction or full mortality of *Chaetomorpha* is significantly higher than previously studied in *C. taxifolia* strands (Odom and Walters 2014). Moreover, chlorine bleach is not effective in inducing full mortality among the macroalgae, as was the case for California's chemical eradication of *C. taxifolia* (Williams and Schroeder 2004). Application of fluridone and copper sulfate is also futile to induce full mortality (Odom and Walters 2014). Acetic acid is one of the only acids tested to consistently reduce survivorship and growth, documented to cause full mortality at 2% concentration for 4-minutes exposure time and 4% concentration acetic acid for 1 minute (Odom and Walters 2014). However, no other dose-responses have been tested.

Given that acetic acid is the most effective tested chemical at causing consistent mortality, the goal of this project is two-fold. The first goal is to determine the minimum concentration and exposure time necessary for full mortality by testing a wide range of doses and responses. The second goal is to test if store-bought acetic acid (vinegar) could replace experiment-grade acetic acid for household hobbyist use.

MATERIALS AND METHODS

Chaetomorpha was purchased from 3 different retailers: LiveAquaria.com (Internet), World Wide Corals (local retail), and Top Shelf Aquatics (local retail). Once purchased, each algal clump was analyzed and placed into a 10-gallon aquarium with 31ppt artificial seawater, the optimal salinity for *Chaetomorpha* (Xu and Lin 2008). All three batches of *Chaetomorpha* were classified as *C. linum*. The artificial seawater was a mixture of deionized water and Instant Ocean sea salts. Each algal batch was exposed to a 12- hour dark/12-hour light cycle for a minimum of one week before the experiments. Pure, experimental-grade acetic acid was purchased from a chemical supply company and concentrations were mixed with 31ppt seawater to determine the minimum effective dose. The trials were conducted at room temperature (25°C) and exposed to standard laboratory lighting of overhead fluorescent bulbs (maintained average illuminance: 400 lux).

C. linum was cut into 1 and 10 cm fragments with a single edge razor blade. The fragments were cut on wet paper towels moistened with salt water to prevent contamination and dehydration. Once cut, the fragments were quickly placed into 55 mm diameter sterile plastic petri dishes with the appropriate acetic acid concentration. Controls were placed in sterile plastic petri dishes with artificial seawater. Forceps were used to transport fragments. The fragments submerged in acetic acid concentrations remained in closed petri dishes for the planned exposure time.

Once the exposure time was reached, each fragment was removed from the petri dish, submerged in deionized water for 5 seconds, and placed in a clean petri dish with 10ml of 31 ppt artificial seawater. After the trial, petri dishes were randomized throughout the laboratory and exposed to 24hour/day light cycle and tracked for survival over time.

Trials 1 and 2

Trials 1 and 2 tested a total of 1800 fragments (900 X 1 cm, 900 X 10 cm) of *C. linum* against 5 concentrations of acetic acid and 9 exposure times. Survival was tracked at 24 hours, 48 hours, 2 weeks, and 5 weeks as shown in Table 1.

Trial 3

Based on the results of Trials 1 and 2, we conducted Trial 3 to narrow the effective concentration range (Table 2). For Trial 3, we tested 360 fragments (10 cm) of *C. linum* against 6 treatments of acetic acid at 6 exposure times. Survival was tracked for 72 hours.

Trial 4

Based on the results of Trials 1 – 3, we conducted Trial 4 to test the efficacy of store-bought acetic acid (cooking vinegar) on fragment survival. 60 fragments (10 cm) of *C. linum* were tested with 5 different commercial vinegars (5-6% diluted acetic acid) at 2 exposure times (Table 3). Survival was tracked for 24 hours.

Survival in all cases was analyzed using a dissecting microscope. If one single cell was still alive, the entire fragment was considered alive because proliferation remained possible (Odom and Walters 2014). A fragment was characterized as dead when all cell walls were shriveled and detached from the outer membrane. If classified as dead, we disposed of the *C. linum* fragments and petri dishes. If the *C. linum* was still

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alive, the water level and salinity of the petri dishes was maintained throughout the monitoring period by adding deionized water. Once the monitoring was complete, all living fragments were disposed of and water from petri dishes was strained with a 1mm mesh net to prevent accidental introduction of any *C. linum* fragments into the environment.

RESULTS

Trial 1

Our results sought out 100% mortality as that is what the aquarium hobbyist industry requires for promotion. No control fragments in saltwater died during the 5-week trial (Table 4, Figure 1). 1% or higher acetic acid, however, caused 100% mortality of both 1 cm and 10 cm fragments after 5 weeks. The lowest dose of 0.05 acetic concentration caused no mortality. Further analysis revealed that a 4% X 30 minute and 2% X 60-minute dose X exposure treatments caused 100% mortality within 24 hours.

Trial 2

Trial 2 was run to replicate Trial 1 and ensure consistency of results. The primary difference between Trials 1 and 2 was that the former found mortality at 1% concentration at 5 weeks, while mortality at the same concentration/week combination did not occur in Trial 2 (Table 5, Figure 1). Again, the 4% X 30 minute and the 2% X 60-minute dose X exposure treatments were effective at killing *C. linum* within 24 hours.

Trial 3

Trial 3 (Table 6) aimed to narrow the concentration range of acetic acid to determine the minimum effective dosage needed to cause 100% mortality in *C. linum* over the short term. The only tested treatment to cause 100% mortality of fragments at 24 hours was 4% acetic acid at 10-min exposure. The other treatment combinations did not cause full mortality, even after 72 hours.

Trial 4

Trial 4 was run to determine a practical way for casual hobbyists to apply acetic acid to rid unwanted *C. linum* from their tanks. All tested commercial vinegars (5-6% acetic acid) caused 100% mortality of all 10-cm long *C. linum* fragments within 24 hours when exposed for 10 minutes (Table 7, Figure 2).

DISCUSSION

After the global invasion of *Caulerpa taxifolia*, public knowledge has increased about the dangers of aquarium dumping. Nevertheless, there is a void in scientifically-based knowledge on proper disposal techniques; many researchers focus on chemical, biological, and mechanical eradication of already invasive species (Odom et al. 2014). In response, we proactively focused on preventing initial introductions by providing hobbyists with a safe, simple, and affordable disposal technique for *C. linum* to preserve the integrity of our ecosystems.

The minimum dosage of acetic acid needed to cause 100% mortality of a 10-cm fragment of *C. linum* within 24 hours was 4% for 10 minutes. In Trials 1 and 2, 2% acetic acid at 60 minutes was also found to eradicate 1 cm and 10 cm fragments within 24 hours. However, we focused on finding a lower exposure time for the convenience of hobbyists. Treating algae at double the concentration for 10 minutes cuts out 50 minutes of exposure, which we believe will encourage more hobbyists to utilize this quick and simple method. We suggest aquarium hobbyist treat their water and any macroalgae fragments with 4% acetic acid, which they can easily access through the use of commercial vinegars. Commercial vinegars range from 4-6% acetic acid and are safe for any household. In order to cause 100% mortality, *C. linum* should be exposed to commercial vinegar for 10 minutes before disposal. For commercial stores and growers, experimental-grade acetic acid can be diluted to 4% solution and may be more cost effective on a larger scale.

Previous studies by Odom and Walters (2014) documented full mortality of *Chaetomorpha* at 2% acetic acid for 4 minutes and 4% for 1 minute. These results were for the eradication of 1 cm fragments with blotted dry, wet weights of 4.1 to 5.7mg (Odom and Walters 2014). In contrast, our experiment focused on 10 cm fragments once they were determined to have higher survivorship than 1 cm fragments in Trials 1 and 2 (Figure 1). Comparison of our results with Odom and Walters's study (2014) shows that larger fragments of *C. linum* may have higher survival rates and require larger concentrations or exposure times to promote complete eradication. When treating larger fragments or clumps of *C. linum*, more research is suggested in order to measure 100% mortality. Large clumps of *C. linum* can additionally be disposed of in a landfill (Odom and

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Walters 2014).

The monitoring time period is also an important factor that accounts for the differences between our experiment and previous studies. While Odom and Walters' results were documented for survival across a 4-week period, our experiment monitored survival after 24 hours. This factor is important because in the wild, a fragment could quickly be introduced into different conditions in each waterway and these conditions cannot be accounted for in a laboratory setting. Nutrients in the natural water may reverse degradation and promote survival. Future studies could also test the immediate and long-term responses of *C. linum* fragments in the wild after treatment with acetic acid.

Utilization of these results is highly recommended due to *Chaetomorpha's* great invasive potential. In addition to having broad environmental tolerances, high nutrient uptake rates, and the ability to reproduce from very small fragments, *Chaetomorpha* also has the tendency to clump together in dense mats (Littler and Littler 2000). When these mats get trapped in benthic communities, they negatively affect the ecosystem by dissolving oxygen and extracting nutrients. *Chaetomorpha* has been documented to have bloom-forming growth in response to eutrophication in nutrient rich water (Lapointe 1997). Dense filamentous clumps and blooms can block sunlight, which will negatively affect the photosynthesis of coastal species. More research is thus required to discover the full effects of *Chaetomorpha* in a variety of ecosystems.

These results could be a tool for government and park officials in the case of future invasion. If the results are used for a potential invasive clean up, I recommend further research on the effects of acetic acid on the ecosystem. A concentration of acetic acid equal to or greater than 5% has been documented to have negative consequences on estuary species (Locke et. al 2009). Since this experiment recommends 4% at 10 minutes for full mortality, additional research should be done on measuring the effects of lower concentrations and more extended exposure times in affected waterways.

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APPENDIX A

Table 1: Survival Tracked at 24 hours, 48 hours, 2 weeks, and 5 weeks

		Acetic Acid Concentration (%)				
Exposure Time (Minutes)		0	0.05	1.0	2.0	4.0
	0	1 cm and 10 cm long fragments 10 replicates of each length, exposure X concentration combination				
	1					
	2					
	3					
	4					
	5					
	10					
	30					
	60					

Table 2: Survival Tracked at 24 hours, 48 hours, and 72 hours

	Acetic Acid Concentration (%)						
Exposure Time (minutes)		0	2.0	2.5	3.0	3.5	4.0
	0	10 cm long fragments 10 replicates each exposure X concentration combination					
	1.0						
	2.5						
	5.0						
	7.5						
	10.0						

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Table 3: Survival tracked for 24 hours.

Exposure Time (minutes)		Commercially Available Vinegar				
		Publix	White House	Heinz	Market Pantry	Heinz All Natural
	0	10 cm long fragments 10 replicates each combination				
	10					

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APPENDIX B

Table 4

<table>
<tr><td rowspan="2"></td><td></td><td colspan="5">Acetic Acid Concentration (%)</td></tr>
<tr><td rowspan="11">Exposure Time (Minutes)</td><td>0</td><td colspan="2">0</td><td>0.05</td><td>1</td><td>2</td><td>4</td></tr>
<tr><td>0</td><td colspan="2" rowspan="10">Living Fragments</td><td colspan="3">Living Fragments</td></tr>
<tr><td>1</td><td rowspan="8" colspan="3">100 % mortality after 5 weeks</td></tr>
<tr><td>2</td></tr>
<tr><td>3</td></tr>
<tr><td>4</td></tr>
<tr><td>5</td></tr>
<tr><td>10</td></tr>
<tr><td>30</td><td rowspan="2">5 weeks</td><td>5 weeks</td><td rowspan="2">24 hours</td></tr>
<tr><td>60</td><td>24 hours</td></tr>
</table>

Table 5

<table>
<tr><td rowspan="2"></td><td></td><td colspan="5">Acetic Acid Concentration (%)</td></tr>
<tr><td rowspan="11">Exposure Time (Minutes)</td><td>0</td><td colspan="3">0</td><td>0.05</td><td>1</td><td>2</td><td>4</td></tr>
<tr><td>0</td><td colspan="3" rowspan="10">Living fragments</td><td colspan="3">Living Fragments</td></tr>
<tr><td>1</td><td rowspan="6" colspan="3">100% mortality after 5 weeks</td></tr>
<tr><td>2</td></tr>
<tr><td>3</td></tr>
<tr><td>4</td></tr>
<tr><td>5</td></tr>
<tr><td>10</td><td>5 weeks</td><td>24 hours</td></tr>
<tr><td>30</td><td colspan="3" rowspan="2">24 hours</td></tr>
<tr><td>60</td></tr>
</table>

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Table 6

	Acetic Acid Concentration (%)						
		0	2	2.5	3	3.5	4
Exposure Time (minutes)	0	Living Fragments					
	1						
	2.5						
	5						
	7.5						
	10	Living Fragments				100% mortality after 24 hours	

Table 7

Exposure Time (minutes)	Commercially Available Vinegar					
	Publix	White House	Heinz	Market Pantry	Heinz All Natural	
0	Living Fragments					
10	100% mortality after 24 hours					

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APPENDIX C

Figure 1

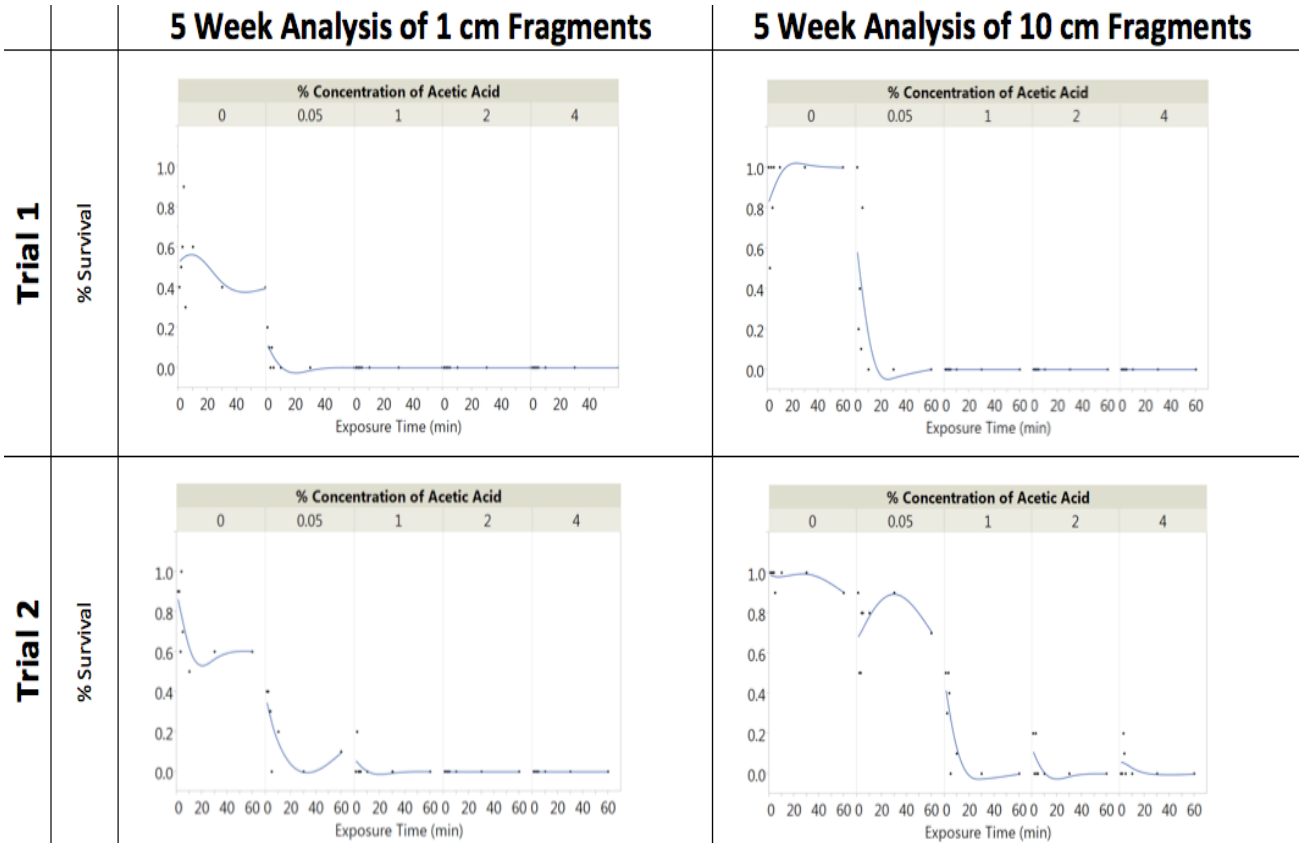

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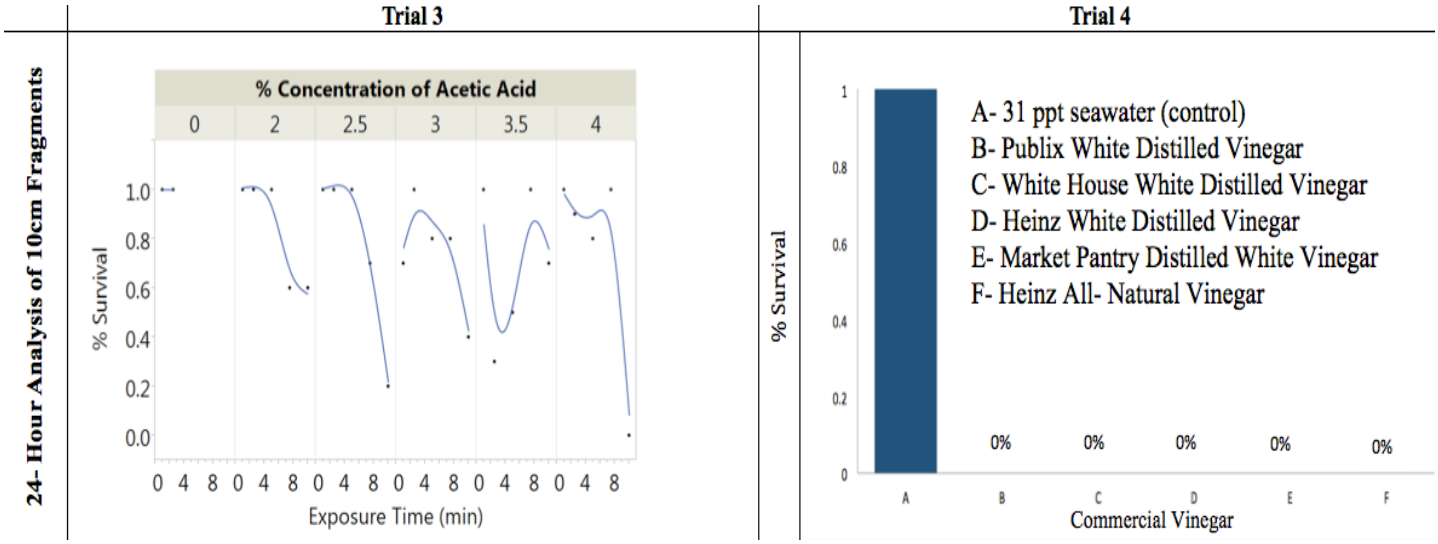
Figure 2



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