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Research Article

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Ecofriendly Bioremediation of Water: Repurposed Macroalgae Removes Eliminates and Neutralizes Heavy Metals

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Abstract
As pollutant contamination in water becomes more and more common, the adverse effects of water pollution is increasingly perceptible, as demonstrated by the destruction of various aquatic ecosystems and pollutant-caused illnesses in humans. Thus, it is pertinent to discover a cost-effective and efficient method that is able to reduce concentrations of water-based pollutants. Current systems in place to remove heavy metal contaminants from the water include chemical chelation, coagulation, and ion exchange, but each have their own pitfalls and are all relatively expensive. Previous research has demonstrated that specific species of seaweeds can absorb water-based pollutants; however, there are limited studies that have tested whether certain species of seaweeds can naturally metabolize and thus neutralize such pollutants after being absorbed. Therefore, this study aims to study the seaweed Sargassum and its ability to metabolize and neutralize iron, a common heavy metal water-based pollutant. Sargassum seaweed was cultured with and without iron, and at varying concentrations of iron, for two weeks to allow for Sargassum growth and thus absorption of the iron. A brine shrimp lethality bioassay was used to compare the cytotoxicity of the water treated with Sargassum against water untreated with Sargassum, thus indirectly measuring relative iron toxicity and concentration. Artemia cysts were put into 24 well plates, and the hatch rate and survival rate of Artemia was calculated in 12-hour increments up to the 48 hour mark. The brine shrimp lethality bioassay demonstrated that Artemia within Sargassum treated water have an increased hatch rate and survival rate compared to Artemia within water untreated with Sargassum. Artemia fed with Sargassum also demonstrated similar survival rates as Artemia within Sargassum treated water. This indicates that Sargassum can absorb iron contaminants from the water and naturally metabolize the iron into a non-toxic form. As Sargassum is readily cheap to obtain and grow, Sargassum serves as a promising cost-effective and efficient alternative to conventional methods of water-based pollutant absorption.

Keywords: Water Pollution, Macroalgae, Sargassum, Cost-effective, Bioremediation, Heavy Metals, Artemia Bioassay

Introduction

Heavy metals have been an increasing problem for our environment in the past century and have begun to negatively impact human health to an alarming degree. Currently, heavy metals are used in numerous domestic, agricultural, medical and technological industries (Tchounwou et al, 2012). This has led to heavy metals being distributed throughout the environment. The most common source of heavy metal contaminants are from the overuse of pesticide and fertilizers in both residential and industrial areas that eventually drains into aquatic ecosystems, such as lakes or rivers (Bashir, 2020). The accumulation of heavy metals, such as iroin, arsenic, cadmium, lead, and mercury, are highly toxic to human health (National Institute of Health, 2017).

Although heavy metals are crucial for the maintenance of organisms, high concentrations in any organism can lead to the impairments of body function and even death (National Institute of Health, 2017). For example, an excess amount of heavy metals in humans can cause vomiting, respiratory tract cancer, kidney diseases, and anemia. High concentrations of heavy metals also impact aquatic ecosystems. It has been long known that marine ecosystems are highly affected by heavy metals (National Institute of Health, 2017). For example, in 1996, decapod crustaceans in their marine ecosystem were found to have the same amount of zinc concentration as the water around them, suggesting that they regularly absorb the heavy metal in the water.

The increased zinc concentration within the decapod crustaceans has been shown to disturb their oxygen levels, reproductive processes, and ultimately decreased their longevity (Bryan, 1996). Furthermore, heavy metals can have widespread ecological impacts, as certain heavy metal pollutants can be bioaccumulated, resulting in increased accumulation of heavy metals in the upper trophic levels of the aquatic food chain. These toxins, which can bioaccumulate in seafood regularly consumed by humans, end up threatening human health (Bashir, 2020).

Currently, there are several methods to reduce the concentration of heavy metals in the environment. One such example is chelation, which removes heavy metal contaminants through a procedure involving the strength of chemical bonds between chelators and metal ions, and the solubility of the chelate in water and lipids (Sears, 2013). Complex processes like chelation may be effective, but are not efficient, nor cost-effective in the long term to be implemented in global aquatic environments or water-treatment facilities, especially when an enormous volume of water has to be treated. As such, new solutions must be found that are cost-effective and efficient in large volumes of water.

Seaweed has shown in previous studies to have characteristics that suggest their capabilities of rapidly absorbing contaminants from their environment. In the past, Neori, Ragg and Shpigel (1998) showed that macroalgae cultivation in residual aquaculture waters can store large amounts of nutrients. Likewise, the *Gracilaria* species of seaweed are able to remove organic nutrients from the water (Marinho-Soriano, Carneiro, Dunes and Peirea, 2009).

Maintaining the toxic material is another characteristic of seaweed. Bryan (1969) expands on this research by testing the effects of the fucoidan (sulfated polysaccharide found in the cell walls of many species of brown seaweed) and absorption in algae. This study found that the fucoidan layer is bound to the zinc which is capable of ion exchange, but once it is absorbed, very little can be lost because it is so tightly connected with the polysaccharides and proteins.

Brine shrimp lethality bioassays are commonly used to measure the toxicity of various chemicals (Apu et al, 2010). This method is not only extremely cheap, but also easy to use at large scales, as the brine shrimp *Artemia* do not need lots of space nor specifically regulated environmental conditions. Taking into account that brine shrimp are extremely sensitive to the toxicity of various chemicals, the brine shrimp lethality bioassay was used in this research.

The purpose of our research was to study the potential of seaweed as a cost-effective and efficient way to absorb and metabolically neutralize contaminants in the water. We hypothesized that one, the longer the *Artemia* is in an environment with iron pollutants and seaweed, the greater the mortality rate and two, compared to *Artemia* in an environment with iron pollutants that have not been treated with seaweed, Artemia in an environment with iron pollutants that have been treated with seaweed will have a lower mortality rate, and thus, an increased survival rate.

Methods

Variables

Two independent variables were implemented - the concentration of iron in water in 1 mg, 5 mg, 10 mg, 40 mg concentrations and the amount of time in 12 hour intervals upto 48 hours. The dependent variables measured were the hatch rate of *Artemia* and the survival rate of *Artemia.*

Outside variables were controlled to the best of our ability. Due to restriction from COVID-19, two of the same set of experiments in two different locations were run. However, external environmental factors were accounted for as much as possible. Lighting conditions were similar for both growing seaweed and hatching the artemia in the different locations. Temperature and humidity were also controlled.

Experiment One

Eight containers were separated into two groups with four containers each: group A and group B. Both groups contained 400mL of water, however, only Group A containers contained seaweed, while Group B containers did not contain seaweed. Finally, for each cup in both groups, we put various concentrations of iron, ranging from 1mg, 5mg, 10mg, and 40mg. Cups with 1 mg of iron concentration were labeled as '1', cups with 5mg of iron concentration were labeled as '2', cups with 10mg of iron concentration were labeled as '3' and cups with 40mg of iron concentration were labeled as '4'.

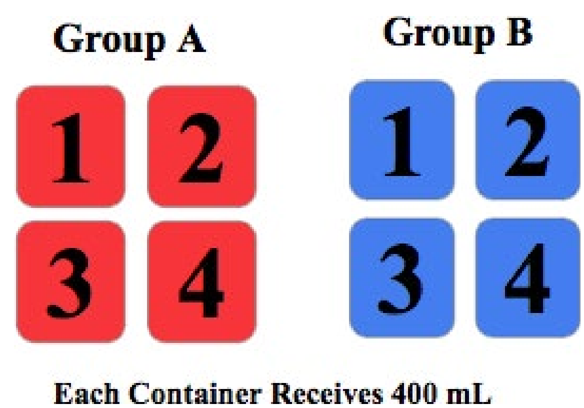

Figure 1: Organization of Containers Utilized.

A 24-well plate was used to hatch and grow the *Artemia*.Two mL of water and ten *Artemia* eggs were placed into each well. Wells in column 6 were not used. Seaweed was added based on the corresponding iron concentration in different columns. Column one contained no iron concentration, and therefore, was our negative control for the experiment.

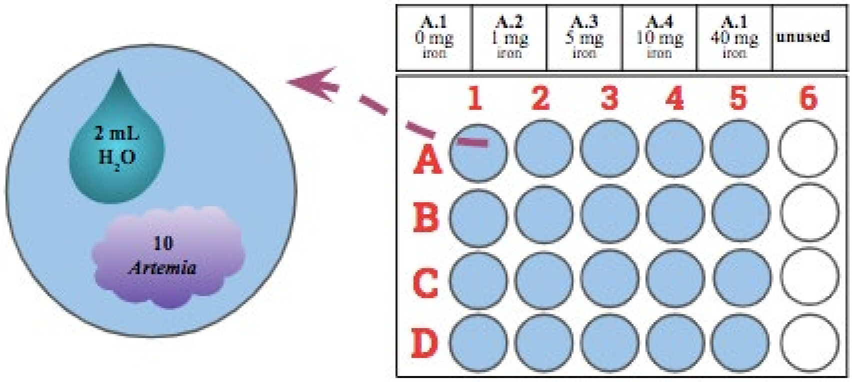

Figure 2: Organization of the 24 well plate.

Experiment Two

Two 24 well plates were utilized. The first 24 well plate contained two mL of water from Group A, in which the seaweed was growing. The second 24 well plate contained two mL of water from Group B, in which the seaweed did not grow. Five *Artemia* eggs were then added into each well for both 24 well plates.

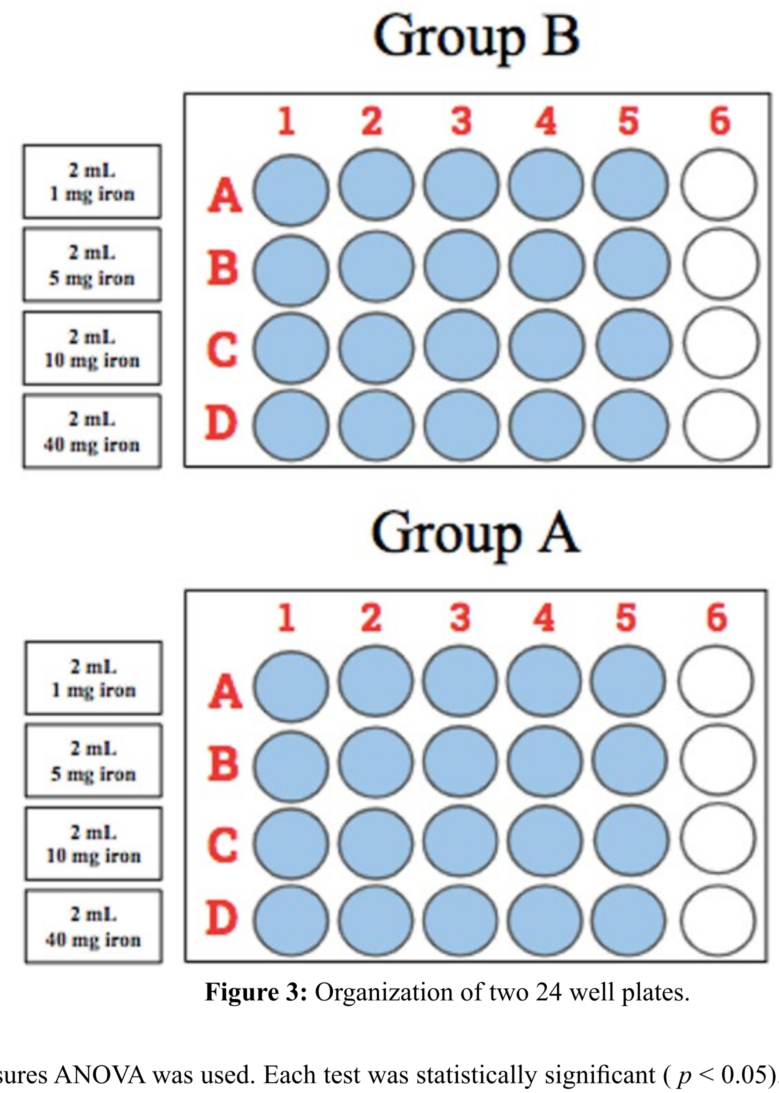

Figure 3: Organization of two 24 well plates.

Results

A repeated between measures ANOVA was used. Each test was statistically significant ($p < 0.05$). At 12 hours, *Artemia* in the highest concentration of iron had the lowest survival rate (represented by the green bar), as shown in figure 4. Generally, survival rates of *Artemia* within the first 12 hours were extremely low, as many *Artemia* did not hatch yet.

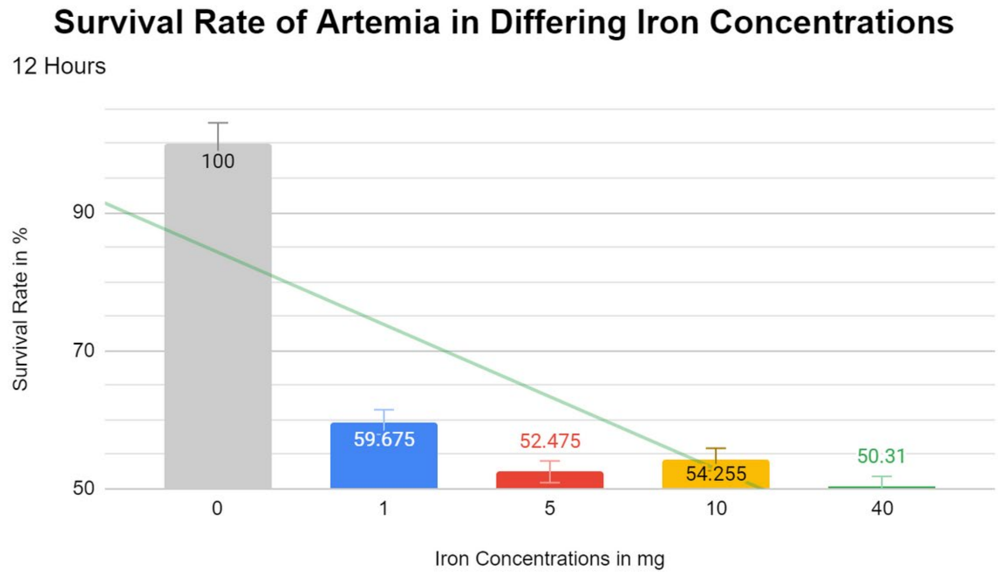

Figure 4: Survival rate of *Artemia* in differing iron concentrations at 12 hours. ($p<0.05$)

At 24 hours, *Artemia* in the highest concentration of iron had the lowest survival rate (figure 5). This follows the same trend as the survival rate of *Artemia* at the 12-hour interval.

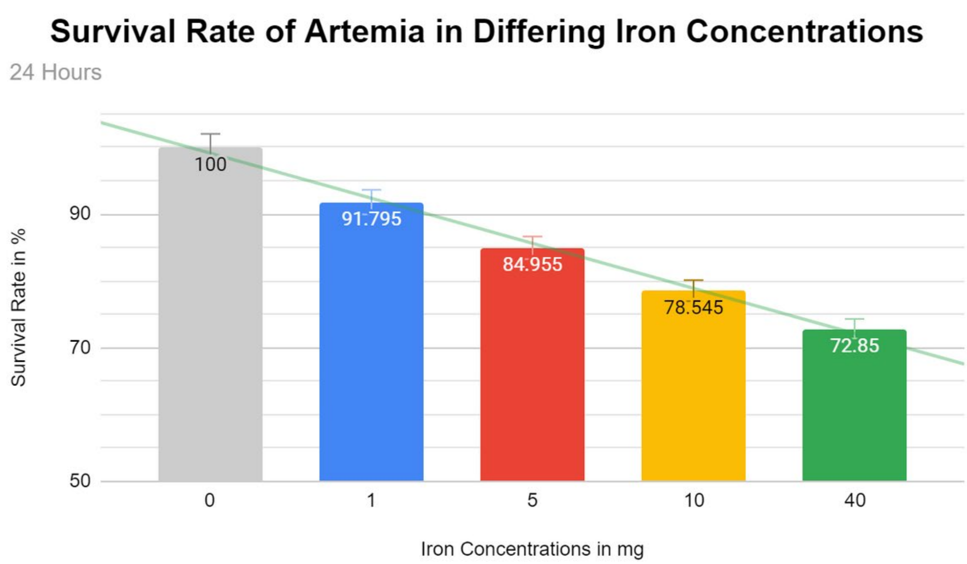

Figure 5: Survival rate of *Artemia* in differing iron concentrations at 24 hours. ($p<0.05$).

The pattern of the lowest survival rate being found in the group with the highest concentration of iron is also present at 36 hours (figure 6).

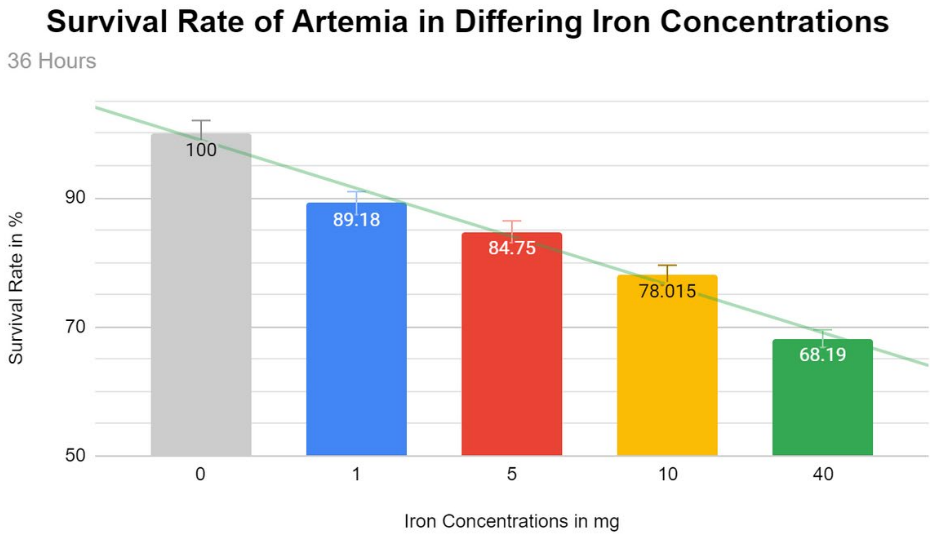

Figure 6: Survival rate of *Artemia* in differing iron concentrations at 36 hours. (p<0.05)

This pattern is consistent at 48 hours as well, with the *Artemia* in the highest iron concentration having the lowest survival rate (figure 7).

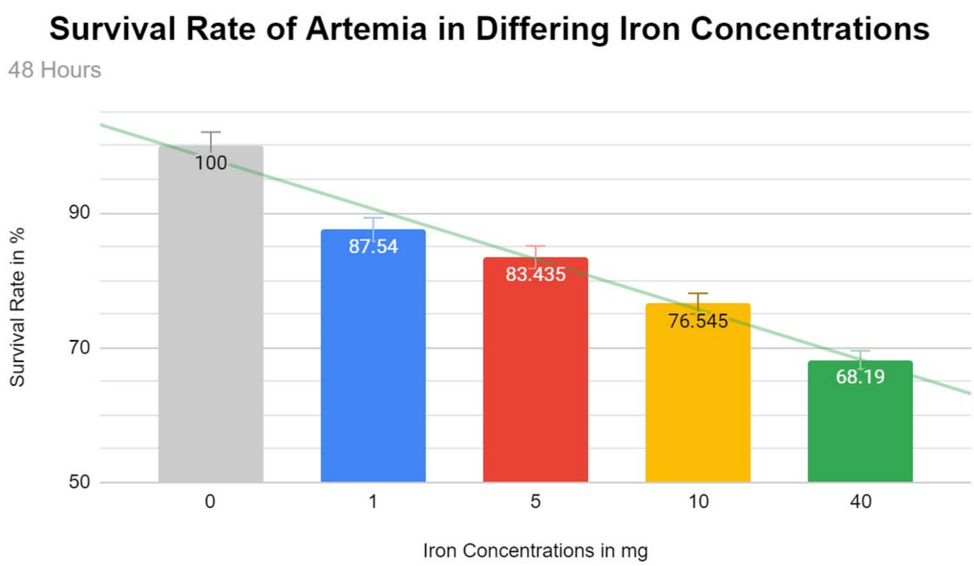

Figure 7: Survival rate of *Artemia* in differing iron concentrations at 36 hours. (p<0.05)

We also combined figures 4, 5, 6, and 7 together to represent the change of the *Artemia* survival rates over a 48 hour time period. This clearly demonstrates how extended exposure to iron led to decreased survival rates across all the iron concentrations. In figure 8, the blue line represents the *Artemia* in the lowest concentration of iron, and you can see that it had the highest survival rate consistently throughout the 12, 24, 36, and 48 hour mark. Similarly, the green line represents the *Artemia* with the highest concentration of iron, and it shows that it had the lowest survival rate.

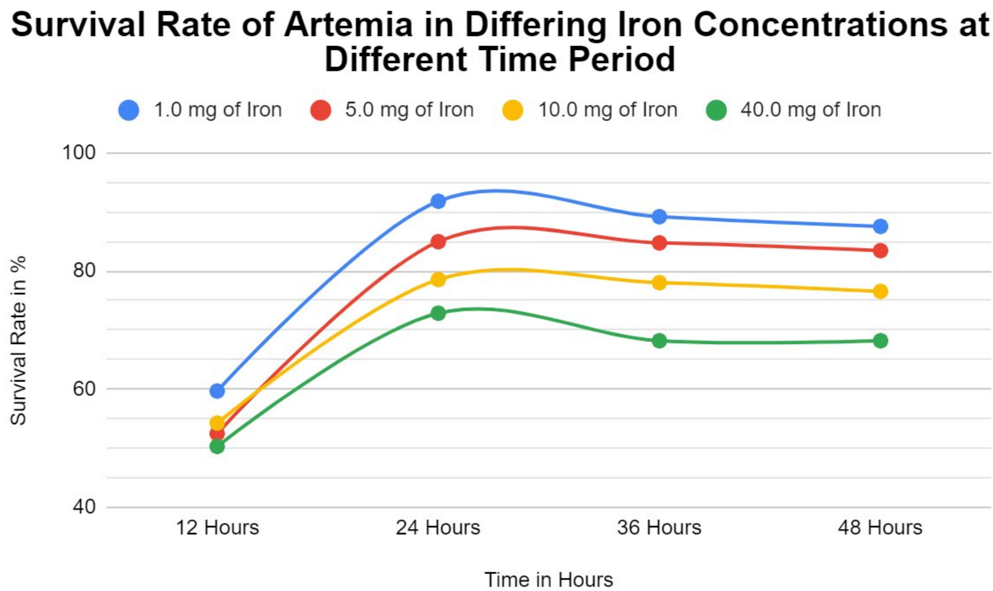

Figure 8: Survival rates of *Artemia* in differing iron concentrations at four different hour marks. ($p<0.05$)

In figure 9, hatch rates of the *Artemia* in Experiment one were graphed. *Artemia* in the lowest concentration of iron had the greatest hatch rate while the Artemia in the highest concentration of iron had the lowest hatch rates. Thus, iron affects the hatching rate of the *Artemia*, which can be implicated in humans and other organisms as well.

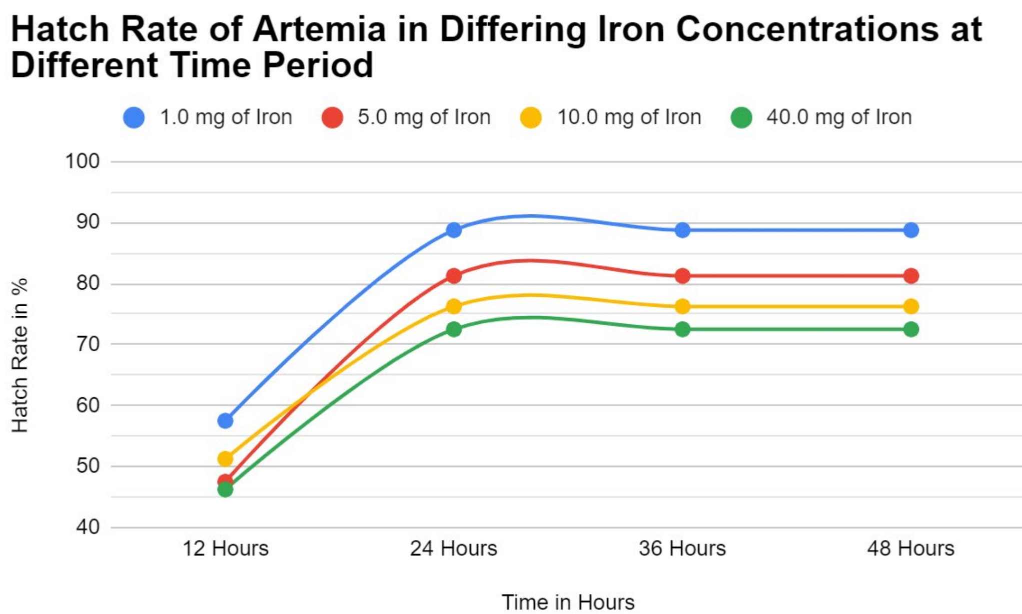

Figure 9: Hatch rates of *Artemia* in differing iron concentrations at four different hour marks. ($p<0.05$)

Experiment two of the method has the purpose of finding the effectiveness of *Sargassum*'s neutralizing ability. From the data, figure 10 demonstrates that *Sargassum* does have a neutralization ability, meaning it did increase the survival rates of *Artemia* in all cases. The blue bar plots the survival rate of artemia in 1.0mg of iron, treated with and without the seaweed sargassum. Similarly, the red bar plots the survival rate of artemia in 5mg of iron, treated with and without the seaweed sargassum. The yellow bar represents 10mg of iron concentration, the green bar represents the 40mg iron concentration, and the gray bar is our control for this experiment.

From our graph, *Artemia* that was grown in an iron polluted environment that was treated with seaweed all had higher survival rates than artemia grown in an iron polluted environment that was not treated with seaweed. Thus, this result demonstrates that seaweed effectively metabolized and neutralized the iron.

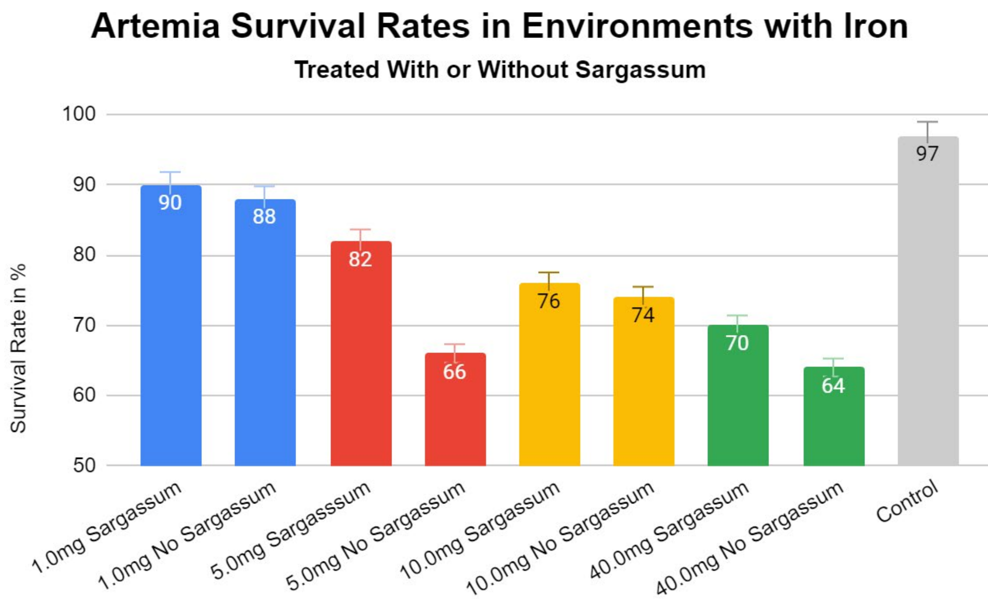

Figure 10: Survival rates of *Artemia* in differing iron concentrations that have or have not been treated with *Sargassum*. (p<0.05)

Discussion

In figure 5 through figure 8, the graph shows how an increased iron concentration relative to normal values demonstrated significant impacts to Artemia survival and hatch rates. As the iron concentration increased, the survival rate and hatch rate of the *Artemia* was negatively affected. Additionally, the longer the *Artemia* was exposed to the iron, the greater the effect iron had on the organism. This could be implicated in humans, as constant micro-exposure to these pollutants, such as through drinking water or ingestion of contaminated foods could lead to increased negative effects on human health. As the hatch rate of Artemia was also affected, iron might also play a role in affecting the health of a child. This could lead to developmental disruptions within the brain or other organs. In figure 9, In figure 10, the graph shows the survival rate of Artemia in differing concentrations of iron, which are represented by the blue, red, yellow, green, and gray bars, under conditions where the water has been or has not been treated with seaweed. As expected, an increase in concentration of iron resulted in decreased survival rates for Artemia across all conditions.

However, the significance of this data demonstrates that *Artemia* living in conditions where the water have been exposed to the seaweed Sargassum resulted in higher survival rates compared to the Artemia living in conditions where the water have not been exposed to the seaweed. From the data, the seaweed Sargassum does indeed confirm our hypothesis that it would absorb and neutralize contaminates within their environment, as shown by the increased survival rates of Artemia.

Conclusion

The results show that *Artemia* in an environment with iron that has been treated with seaweed had a greater survival and hatch rate than Artemia in an environment with just iron. This demonstrates that seaweed has a natural metabolic process that can neutralize the iron. The toxic iron is basically metabolized into a safer form of iron for the seaweed to use - thus, if the seaweed secreted iron, the iron would be less harmful to the environment and the organisms within them. Therefore, this has huge implications for cleaning municipal drinking water systems, as it has the potential to remove dangerous contaminants from the water much more rapidly and effectively than other chemicals used. It can also be put into use in natural environments, as seaweed has the potential to remove toxins being released into the oceans by human activities and toxins secreted by other organisms. For example, an oil spill can quickly be absorbed by the seaweed.

Limitations

There were environmental differences such as temperature fluctuation that we could not control; future experiments can be conducted in a lab setting where temperature can be manipulated.

Only one type of contaminant (iron) was tested; future experiments should look at a combination of contaminants since polluted aquatic environments in reality have a combination. Furthermore, only two rounds of the experiment were done. Future extensions should repeat trials multiple times to determine consistency.

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Statements and Declarations

Ethical Approval
Roslyn High School's Scientific Review Board approved the research proposal.

Consent to Participate
Not applicable.

Consent to Publish
Both authors consented to publishing.

Author Contributions
All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Jessie Dong and Maxx Yung. The first draft of the manuscript was written by Jessie Dong and afterwards, both authors edited this version of the manuscript.
Both authors read and approved the final manuscript.

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Competing Interests
The authors have no relevant financial or non-financial interests to disclose.

Availability of Data and Materials
Not applicable.

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