\*NOTE: As this was a group project, a notebook could not be utilized. A notebook would have not been COVID-19 safe.

<https://www.cysf.org/wp-content/uploads/logbooks.pdf>

<https://www.cysf.org/what-to-expect/>

Tentative Timeline:

December 29 - January 20

* Come up with ideas and complete basic research to get a good idea of what we are doing
* Complete background

January 20 - 31

* Source materials, complete procedure, complete materials list

March 1 - 9

* Complete experiment and raw data tables for qualitative and quantitative data
* Complete processed data tables and statistical analysis

March 10-17

* Complete conclusion and extensions
* Upload everything on CYSF platform
* Start creating video presentation

March 17-19

* Final edits and submit

|  |  |  |
| --- | --- | --- |
| Date | Progress | Ideas and Problems |
| December 29 | Began research to decide what science fair project would be on | * Bioplastic
	+ Had previously done it before, not very interesting
* Biofuel
	+ Expensive, needs special equipment, dangerous
* Spinach decellularization
	+ Expensive and needs special equipment
* Heavy metal filtration
	+ Not much information regarding this
 |
| January 10 | Narrowed down ideas to spinach decellularization and heavy metal filtration using seaweed | * Spinach decellularization takes a long period of time
* Not much research about heavy metal filtration using seaweed
 |
| January 17 | Decided on heavy metal filtration using seaweed and started background research. Deciding between zinc tablets and iron tablets for heavy metal.  | * Using tablets would allow for more control for concentration in water
* Iron is easily accessible
* Both are not soluble
 |
| January 21 | Decided to use iron vitamin tablets for heavy metal and brown seaweed for the type of seaweed. Planning to ask the school for an iron level indicator when semester two begins. Created a **materials** list. | * Chelated iron tablets
* Kelp seems effective in biosorption
* Emailed Mrs. Ozero for iron indicator
* Materials list https://docs.google.com/document/d/1AzYIfZkHAn8MvRGogJTO5FvQZgBfhYvVo1nFHEHyrRs/edit?usp=sharing
 |
| January 29 | Began sourcing materials. Went to Emart and Walmart. | * Bought iron caplets and seaweed
 |
| February 3 | Got aquarium iron testing kit from Mrs. Ozero | * Aquarium testing kit - limited range of concentrations it can test for
 |
| Febuary 8 | Started to plan out details about the structure and **procedure** of the lab, wrote **variables** | * Will complete in home
* Sauhe has sufficient apparatus + materials to do this
* Variables were straight forward - will do 5 trials for utmost accuracy
* Recorded here https://docs.google.com/document/d/1AzYIfZkHAn8MvRGogJTO5FvQZgBfhYvVo1nFHEHyrRs/edit?usp=sharing
 |
| February 11 | Conducted **preliminary testing**Found out the species of kelp we had purchased:**Saccharina japonica** | * Iron caplets are chelated so it dissolves, however, chelation is part of the biosorption process so we cannot use it
* This caplet is specifically chelated with amino acid - ferrous bisglycinate
 |
| February 13 | Completed **background information** | * Citations done APA - in text citations used
* Recorded in https://docs.google.com/document/d/1AzYIfZkHAn8MvRGogJTO5FvQZgBfhYvVo1nFHEHyrRs/edit?usp=sharing
 |
| February 19 | Completed **final materials list** and **procedure** + wrote a **hypothesis** | * Had all materials needed
* Wrote hypothesis - but was the wrong format
* Fixed hypothesis
* Written here https://docs.google.com/document/d/1AzYIfZkHAn8MvRGogJTO5FvQZgBfhYvVo1nFHEHyrRs/edit?usp=sharing
 |
| February 20 | Conducted **trials 1-3** and obtained **qualitative data** on **data table 1**Used color indicator chart to log **quantitative data** on **data table 2** | * Worked very successfully
* Color indicator chart was limited, however, worked as well as it could
* Logged here https://docs.google.com/document/d/1AzYIfZkHAn8MvRGogJTO5FvQZgBfhYvVo1nFHEHyrRs/edit?usp=sharing
 |
| February 21 | Conducted **trials 4 and 5** and obtained **qualitative data on data table 1**Used color indicator chart to log **quantitative data on data table 2** | * Successful experimentation again
* Logged here https://docs.google.com/document/d/1AzYIfZkHAn8MvRGogJTO5FvQZgBfhYvVo1nFHEHyrRs/edit?usp=sharing
 |
| February 26 | Decided on **Two-Tailed Pearson Correlation Test** | * Had previously been used for IA
* Decided not to do a skewness test as correlation was already very significant
 |
| February 27 | Completed **statistical analysis** and **graphing** | * Graphs uploaded into google docs
* Completed https://docs.google.com/document/d/1AzYIfZkHAn8MvRGogJTO5FvQZgBfhYvVo1nFHEHyrRs/edit?usp=sharing
 |
| February 28 | Completed **conclusion + extension** | * Since seaweed is plentiful and easily accessible, would be good to create economical heavy metal filters
* Written here https://docs.google.com/document/d/1AzYIfZkHAn8MvRGogJTO5FvQZgBfhYvVo1nFHEHyrRs/edit?usp=sharing
 |
| March 6 | Uploaded everything on **CYSF Platform** | * No space for logbook? So we will most likely upload in the attachments
 |
| March 7  | Review to ensure that everything is correct on CYSF platform | * Small grammar edits
 |
| March 13 | Began making a slide show for **video presentation** | * Made powerpoint and decided to use CloudApp
* Or Veed
	+ <https://www.veed.io/screen-recorder>
 |

**Background**

Iron is an essential element for nutrition in pro- and eukaryotic cells. A majority of iron in the body is present as hemoglobin, myoglobin, heme-containing enzymes, or stored as ferritin (Saito, 2014). When the body absorbs an abundance of iron and its iron binding proteins are oversaturated, the excess iron is stored in major organs such as the liver (E Guarin, 2020). The stored may result in damage to healthy cells such as skin and internal organs.

In nature, iron is rarely found in its elemental form; most commonly in oxide form (Cameron, n.d.). Iron is found in abundance in nature and naturally found in low concentrations in water, usually around 0.5-10 mg/L (Health Canada, 2009). Although drinking water contains less than 0.3 mg/L, numbers may be higher in areas that utilize cast iron or steel pipes (WHO, 2007). Numbers also may be higher in areas that use iron salts as coagulating agents in the water purification process (IWA Publishing, 2010).

Various species of macroalgae, commonly known as seaweed or kelp, such as *Saccharina japonica,* are known for their effective biosorption. This allows for heavy metals such as Cadmium, Iron, Mercury, and Arsenic to bind to the cell wall of marine algae and seaweed (Ibrahim, 2011). The efficacy of the biosorption of macroalgae are primarily due to sulphated polysaccharides ─ negatively charged polysaccharides that are present in the cell wall or macroalgae (Ali Redha, 2020).

The complex process of biosorption in Saccharina Japonica goes through several mechanisms: transportation across cell membrane, physical adsorption, ion exchange, complexation, and precipitation (Ahalya et al., 2003). Physical absorption is mediated through electrostatic interactions (Van der Waal forces) between the cell microbes and the metallic ions in the solution. Such intermolecular forces entail London dispersion (momentarily induced dipole forces), dipole-dipole forces of polar molecules, ion-dipole force (ion and partial charge of molecule) and lastly hydrogen bonding forces (Ahalya et al., 2003). Complexation entails the formation of a complex from insoluble ions through the interaction with carboxyl and amino acid groups (both chelation agents) (Schiewer, 1999).

For the experiment, a rusty nail will be used to introduce iron ions into water. Rust is a hydrate that is formed through an oxidation reaction. It is a hydrated form of ferric oxide, the approximate compound being Fe2O3•32H2O. The balanced chemical equation is as follows (Vitz et al., 2016):

4Fe (s) + 3O2 (g) + 2xH2O (l)→ 2Fe2O3•xH2O (s)

Although hydrates have a low solubility, a small portion dissociates when placed into water into ferric ions and oxygen which would rise to the surface.

 The reagent used to spectrophotometrically quantify the presence of iron (II) in water was the Nutrafin iron reagent test. The test utilizes hydroxylamine hydrochloride and 2,4,6-tripyridyl-s triazine (TPTZ) both in concentrations less than 1% in the reagent. In the presence of hydroxylamine, iron is reduced to iron (II). TPTZ reacts with the iron (II) to form a violet coloured complex. Spectrophotometrically, the absorbance of the complex is measured at approximately 593 nm(*Corn Syrup Analysis E-33- 1 Analytical Methods of the Member Companies of the Corn Refiners Association, Inc*, n.d.).

**Variables**

Controlled:

* Amount of iron added to water
* Volume of water
* Glassware and apparatus type used
* Temperature, air pressure, humidity of environment

Manipulated:

* Time kelp is in solution (0min, 10 min, 20 min, 30 min, 40 min, 50 min, 60 min)

Responding:

* Concentration of iron (III) ions in water

**Hypothesis**

If 10 g of *Saccharina japonica* is added to 100mL of iron (III) solution with a concentration of 1mg/L, then the ferric ion concentration will decrease because of the effective biosorption of macroalgae.

Since a Pearson correlation test is being conducted, a null hypothesis needs to be introduced:

H0: There is no correlation between *Saccharina japonica* and the removal of iron (III) from water.

**Materials and Apparatus**

* 7 plastic test tubes
* Tape and sharpie (for labelling test tubes)
* Nutrafin Iron Testing Kit
* 2 rusty nails
* 100 mL beaker
* Bowl
* 12 g of Dried *Saccharina japonica*
* Distilled water bottle
* Scale
* Mortar and pestle
* Safety goggles
* Glass stirring rods
* 10 mL graduated cylinder
* 100 mL graduated cylinder
* 250 mL beaker
* stopwatch

**Procedure**

1. Place two rusty iron nails in 650.0 mL of distilled water in a bowl for 48 hours.
2. Prepare 7 test tubes and label one as "control." Label the rest of the test tubes with 10 min, 20 min, 30 min, 40 min, 50 min and 60 min.
3. Put 4.50 mL of the solution inside the test tube labelled "control" using a 10 mL graduated cylinder.
4. Put two drops of reagent #1 from the Nutrafin Iron Testing Kit into the test tube. If the concentration is determined to be 1.0 mg/L, proceed to step 4. If the concentration is determined to be lower, let the rusty nails sit in the solution overnight. If the concentration is too high, dilute it with 5.0 -10.0 mL of distilled water accordingly.
5. Remove the rusty nails from the solution.
6. Measure 51.0 g of *Saccharina japonica* on a scale.
7. Ground the *Saccharina japonica* using a mortar and pestle.
8. Measure out 100.0 mL of solution using a 100mL graduated cylinder and pour it into a 250mL beaker.
9. Put 10.0 g of ground *Saccharina japonica* into the beaker with the solution and immediately start the stopwatch.
10. Stir the solution every 2 minutes.
11. After 10 minutes, measure out 4.5 mL of the solution using a 10 mL graduated cylinder and pour in the test tube labelled “10 min.” Do not get Saccharina japonica in the test tube.
12. Put two drops of Iron reagent #1 in the test tube and stir it with a glass stirring rod
13. Repeat steps 9 to 12 for each 10-minute interval for test tubes labelled “20 min,” “30 min,” “40 min,” “50 min,” and “60 min.”
14. After 30 minutes, compare the test tubes to the colour indicator chart and log on Table 1.
15. Repeat steps 9-14 for four more trials.

Table 1: Observations of 30 minutes after adding reagent #1 to test tubes for 5 trials.

|  |  |
| --- | --- |
| Test Tube label | Observations |
| Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 |
| Control | Translucent, deep violet | Translucent, deep violet | Translucent, deep violet | Translucent, deep violet | Translucent, deep violet |
| 10 min | Translucent, medium violet | Translucent, medium violet | Translucent, medium violet | Translucent, medium violet | Translucent, medium violet |
| 20 min | Translucent, medium violet | Translucent, medium violet | Translucent, medium violet | Translucent, medium-light violet | Translucent, medium violet |
| 30 min | Translucent, medium-light violet | Translucent, medium violet | Translucent, medium violet | Translucent, medium-light violet | Translucent, medium-light violet |
| 40 min | Translucent, medium-light violet | Translucent, medium-light violet | Translucent, medium-light violet | Translucent, light violet | Translucent, medium-light violet |
| 50 min | Translucent, light violet | Translucent, light violet | Translucent, light violet | Translucent, light violet | Translucent, light violet |
| 60 min | Translucent, colorless | Translucent, colorless | Translucent, colorless | Translucent, colorless | Translucent, colorless |

Table 2: Concentration of iron (III) in water (mg/L) after 10g ($\pm $0.5 g) of *Saccharina japonica* is added into iron solution, measured every 10 mins intervals over the course of 60 mins.

|  |  |
| --- | --- |
|   | Concentration of iron(III) (± 0.15 mg/L) |
| Time after 10 g ($\pm $0.5 g) *Saccharina japonica* is added to 100 mL ($\pm $0.2 mL) of iron solution (± 1 min) | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 |
| 0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 10 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| 20 | 0.50 | 0.50 | 0.25 | 0.25 | 0.50 |
| 30 | 0.25 | 0.50 | 0.10 | 0.25 | 0.25 |
| 40 | 0.25 | 0.25 | 0.10 | 0.10 | 0.25 |
| 50 | 0.10 | 0.10 | 0.00 | 0.10 | 0.10 |
| 60 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Table 3: Averages of 5 trials of concentration of iron (III) in water (mg/L) after 10g of *Saccharina japonica* is added to iron solution, measured every 10 mins intervals over the course of 60 mins.

|  |  |
| --- | --- |
| Time after 10g ($\pm $0.5 g) *Saccarina japonica* is added to 100 mL ($\pm $0.2mL) of iron solution ($\pm $1 min) | Average concentration of iron (III) (± 0.15 mg/L) |
| 0 | 1.0 |
| 10 | 0.50 |
| 20 | 0.40 |
| 30 | 0.27 |
| 40 | 0.19 |
| 50 | 0.08 |
| 60 | 0.00 |

Since a Pearson correlation test is being conducted, a null hypothesis needs to be introduced:

H0: There is no correlation between *Saccharina japonica* and the removal of iron (III) from water.

Graph 1: Averages of 5 trials of concentration of iron (III) in water (mg/L) after 10g of

*Saccharina japonica* is added to iron solution, measured every 10 mins intervals over the course of 60 mins. Error bars represent uncertainties.



Note: Pearson's R2 value is shown beside the linear regression line.

Equation 1: Equation for the Pearson correlation coefficient (*r*) used in Excel PEARSON function

$r = \frac{n(\sum\_{}^{}xy)-(\sum\_{}^{}x)(\sum\_{}^{}y)}{\sqrt{[n\sum\_{}^{}x^{2}-(\sum\_{}^{}x)^{2}][n\sum\_{}^{}y^{2}-(\sum\_{}^{}y)^{2}}}$

Table 4: Two-tailed Pearson correlation test for the correlation between *Saccharina japonica* and the removal of iron (III) from the water it is in

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Correlation between *Saccharina japonica* and the removal of iron (III0 from water | Degrees of freedom | Significance | Pearson coefficient (*r*) | Critical value |  H0: There is no correlation between *Saccharina japonica* and the removal of iron (III) from water. |
| 7 | 0.05 | -0.93 | 0.754 | |-0.93|>0.754, H0 is rejected. There is a statistically significant correlation. |

Table 5: *t* value and resulting *p* value found of the correlation between *Saccharina japonica* and the removal of iron (III) from water

|  |  |
| --- | --- |
| t | p |
| -5.73 | 0.00185 |

**Conclusion**

Ultimately, the results of the statistical analysis for this investigation support the hypothesis that *Saccharina japonica* will decrease the concentration of ferric ions in the solution. Therefore, the null hypothesis can be rejected as the statistical analysis supports that there is a significant correlation between time after *Saccharina japonica* is added and ferric ion concentration.

The preliminary test with the graph and the coefficient of determination (R2) showed that 86.8% of the y values could be explained by their corresponding x values; percentage variation of y by x. In the Pearson coefficient test, the absolute value of the r value was greater than the critical value; therefore, the null hypothesis was rejected signifying a statistically significant correlation. The p value signifies the probability that the correlation is found in a world where the null hypothesis is true. Since the probability is 0.010 or 1.0%, the correlation found in this experiment is highly significant.

**Sources of Error**

A systematic source of error for this experiment was that the Nutrafin iron indicator test was colorimetric. Since a colorimeter was not utilized, the data was collected through closely comparing the color of the solution with the color indicator chart. Due to this, the accuracy of the data collected was limited. This led to a greater uncertainty in the concentration of ferric ions which created greater room for error. This error could be mitigated by using a colorimeter or by using a spectrophotometer to obtain more accurate readings of the concentration.

Another systematic source of error was that the iron concentration test could only test the presence of up to 1.0mg/L of ferric ions in the solution. This could have led to the concentration of the control being higher than 1. mg/L. This would cause the correlation of the data to be weaker than it actually had been, as it would show a less steep slope between the first point (control) and second point (10 min). This error could be fixed through using a lower concentration than 1.0 mg/L for the control. Additionally, a different iron indicator test could be utilized to ensure that the control value does not exceed 1.0 mg/L. A spectrophotometer could also be utilized to accurately measure the concentration of the solution.

**Application and Extension**

As *Saccharina japonica* has a large biosorption capacity, it could be utilized to filter toxic heavy metals such as mercury and arsenic from water as well. Many water sources in developing countries are contaminated, which increases health risks. Often, water filters are expensive and not accessible for these countries, however, seaweed is abundant and cheap to harvest. Inexpensive filters using seaweed could be produced to prevent deaths and illnesses due to poisoning.

The experiment could be carried out using other toxic heavy metals and different types of macroalgae to explore which species is most effective in biosorption of metals. This could then be used to develop and design accessible and economical filters.

**Citations**

Health Canada. (2009). *Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Iron - Canada.ca*. Canada.ca. https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadian-drinking-water-quality-guideline-technical-document-iron.html

IWA Publishing. (2010). *Coagulation and Flocculation in Water and Wastewater Treatment | IWA Publishing*. Iwapublishing.com. https://www.iwapublishing.com/news/coagulation-and-flocculation-water-and-wastewater-treatment

WHO. (2007). *Iron in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality*. https://www.who.int/water\_sanitation\_health/dwq/chemicals/iron.pdf

Böttger, L. H., Miller, E. P., Andresen, C., Matzanke, B. F., Küpper, F. C., & Carrano, C. J. (2012). Atypical iron storage in marine brown algae: a multidisciplinary study of iron transport and storage in Ectocarpus siliculosus. *Journal of Experimental Botany*, *63*(16), 5763–5772. <https://doi.org/10.1093/jxb/ers225>

E Guarin, G. (2020, September 20). What is the pathophysiology of transfusion-induced iron overload? Www.medscape.com. https://www.medscape.com/answers/1389732-177323/what-is-the-pathophysiology-of-transfusion-induced-iron-overload

Saito, H. (2014). METABOLISM OF IRON STORES. *Nagoya Journal of Medical Science*, *76*(3-4), 235–254. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4345694/

Cameron, D. (n.d.). *Iron - Chemistry Encyclopedia - elements, metal, mass*. Www.chemistryexplained.com. Retrieved March 13, 2021, from <http://www.chemistryexplained.com/Hy-Kr/Iron.html#:~:text=Iron%20has%20seven%20oxidation%20states>

Ibrahim, W. M. (2011). Biosorption of heavy metal ions from aqueous solution by red macroalgae. *Journal of Hazardous Materials*, *192*(3), 1827–1835. https://doi.org/10.1016/j.jhazmat.2011.07.019

Ali Redha, A. (2020). Removal of heavy metals from aqueous media by biosorption. *Arab Journal of Basic and Applied Sciences*, *27*(1), 183–193. https://doi.org/10.1080/25765299.2020.1756177

Ahalya, N., Ramachandra, T. V., & Kanamadi, R. D. (2003, December). *Biosorption of Heavy Metals*. https://www.researchgate.net/publication/257029311\_Biosorption\_of\_Heavy\_Metals

Schiewer, S. (1999). Modelling complexation and electrostatic attraction in heavy metal biosorption by Sargassum biomass. *Sixteenth International Seaweed Symposium*, 593–601. <https://doi.org/10.1007/978-94-011-4449-0_73>

*Corn Syrup Analysis E-33- 1 Analytical Methods of the Member Companies of the Corn Refiners Association, Inc*. (n.d.). Retrieved March 13, 2021, from https://corn.org/wp-content/uploads/2009/12/E-33.pdf