

The Effect of Iron as a Potential Inducer of Cataracts

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I. Abstract

Cataracts are a leading cause of blindness, especially in the developing world. With clean water not always accessible in many third-world countries, the poor quality of water may trigger oxidative stress, a major inducer of cataracts, as demonstrated by previous studies, and one-way oxidation can occur is through Fenton reactions reacting with iron. Perhaps the iron in the untreated water causes oxidation, and this could be a potential cause of cataracts. The objective of the study is to find if the presence of iron ions in drinkable water sources is a contributing factor to cataracts. Pig eyes were soaked in iron solutions and stored under a UV light for 24 hours, simulating human eyes in contact with iron water over a long period, building up oxidative damage. The lenses were then analyzed by comparing the color of the lenses and the change in luminance compared to the control. The results were found to show how iron in water can induce oxidation in the eye due to the Fenton Reaction, leading to cataracts.

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III. Key Words:

Cataracts, Iron, Iron in Water, Fenton Reaction, Photo Fenton Reaction, Fenton Reagent, Photo-Fenton Reagent, Oxidation, Eye, Lens, Inducer of Cataracts

IV. Abbreviations and Acronyms:

UV: Ultraviolet

AOP: Advanced oxidation processes

V. Acknowledgements:

Ms. Renee Fallon, my teacher at Monta Vista High School, was my mentor for this project, and provided guidance, helping me develop research skills. Monta Vista Research Club officers, consisting of other Monta Vista students, helped keep track of my progress and provided me with additional support when I needed it.

VI. Biography

Ethan Liu is a junior at Monta Vista High School in Cupertino, California. His first involvement in a local science fair as a 4th grader sparked his interest in science, and he has been participating in science fairs and conducting research on various biology and health-related topics in the past two years.

Ethan Liu has participated in the Synopsys Silicon Valley Science and Technology Championship to do independent project-based research to address questions in the field of Biochemistry/Microbiology. This year, he received the 1st Award in the Biological Science and Engineering Category & Certificate of Achievement, as well as the opportunity to advance to the 2022 California Science & Engineering Fair.

In his free time, Ethan likes to hang out with friends and plays the piano. He is part of many volunteer clubs, including the American Red Cross (Youth Services Silicon Valley Chapter) and SoundPost Youth Foundation, where he engages in different volunteering service activities and helps prepare and host community events. He hopes to be able to help others and make a positive difference in the world.

1.0 Introduction

Cataracts, the development of opacity within the lens, are the cause of 47.9% of blindness worldwide. “It is estimated that more than half of people over the age of 65 years have some degree of cataract development in one or both eyes”. [12] Blindness is typically treatable and preventable within 75% of these cases, but 90% of blind people live in the poorest places of the developing world leaving them without the medical technology needed for treatment [2].

For an eye to see, light passes through a clear lens. The cornea and the lens help direct the light rays to the retina. The pupil and the iris regulate the amount of light entering the eye, which provides an optimal image. The retina absorbs the light and converts the light to electrochemical impulses, which are transported to the optic nerve and sent to the brain as an image [8].

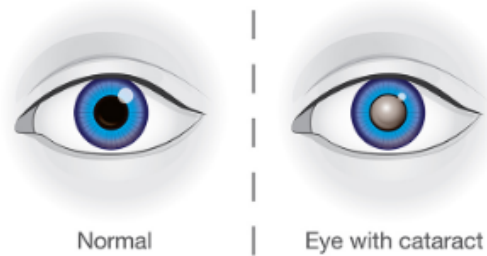


Fig 1. Normal Eye vs Eye with Cataract (Image from AOA)

A cataract is the development of opacity within the lens, where the proteins and fibers of the lenses break down. [4] There are multiple types of cataracts, including Nuclear Sclerotic Cataracts, Cortical Cataracts, and Posterior Subcapsular Cataracts. Oxidative damage is a major cause of cataracts. [3]

1.1 Causes of Cataracts

The main cause of cataracts is age. As humans age, the structure of the lens and accumulation of pigment changes through damage. The clarity of the normal lens is maintained through a precise structural arrangement of fibers and a balance of chemical constituents. Over time, the new fibers on the crystalline lenses build up and are not removed or replaced. This change to the microstructure results in opacification, which consequently alters the penetration and refraction of light. As a result, rather than the light focusing on a single point on the retina, this clouding of the lens will cause light to scatter. The more opaque/cloudy it becomes, the greater the scatter and the worse the vision [12].

There are also other external influences associated with cataract formation including socioeconomic and geographical factors. In third-world countries, malnutrition, acute dehydrating

diseases, and cumulative exposure to sunlight/UV rays contribute to cataract formation. In developed countries, smoking, diabetes, and drug use contribute to cataracts forming [2]. This is most likely due to the oxidative damage occurring. Other causes of cataracts include trauma in a variety of forms, including but not limited to direct penetration, contusion, radiation, electrical, metabolic, and congenital disorders [2].

1.2 Iron's Role in the Formation of Cataracts

Oxidation is a prominent feature of nuclear and cortical cataracts. With age, the lens nucleus becomes more susceptible to oxidation [3].

Oxidation is a natural product of metabolic processes in the body. Normally, the body produces

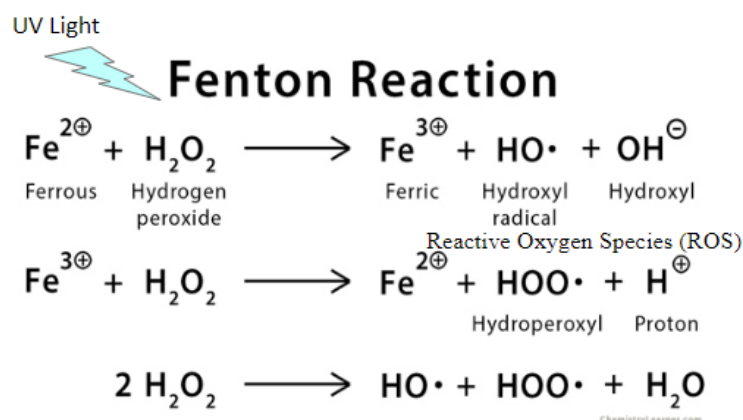


Fig 2. Fenton Reaction (Image from ChemistryLearner.com)

reactive oxygen species to induce healthy apoptosis. There is a balance of free radicals and antioxidants in the body to maintain homeostasis. [15] However, an excess of free radicals, such as oxygen-free radicals ($\text{O}_2\cdot^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\text{OH}\cdot$). Fenton reactions ($\text{H}_2\text{O} + \text{O}_2 \rightarrow \text{H}_2\text{O}_2$, $\text{H}_2\text{O}_2 + \text{Fe(II)} + \text{HO} + \text{HO}\cdot$, $\text{H}_2\text{O}_2 + \text{Fe(III)} + \text{HO} + \text{HO}\cdot$) can increase the rate of oxidative damage [14].

When there is extra iron in the body (hemochromatosis) and underexposure to ionizing radiation (UV rays), the extra iron undergoes the Fenton reaction, where molecules of iron $2+$ are oxidized by hydrogen peroxide, producing iron $3+$ and the hydroxyl radical and hydrogen ion as byproducts [6]. Now, iron $3+$ can be reduced back to iron $2+$ via hydrogen peroxide again, creating a peroxide radical and a protein. This cycle repeats, like an endless loop. Over time, free radicals built up during the immune response as a result of the Fenton reaction damages cells, which can cause cell, DNA, and protein damage [17].

The cytoplasm of the cells inhibits oxidation in the eye. SOD, an enzymatic antioxidant, protects the cells by taking the O_2 and converting it into H_2O_2 . [18] Glutathione Peroxidase, an enzyme that catalyzes the reduction of hydrogen peroxide to water and oxygen, making it benign, goes through the lipid hydroperoxide in the aqueous phase of the cell membrane. [11] This protects the cell membrane

from the damage caused by the Fenton reaction. However, with age, the SOD levels decrease. The decreased SOD levels may lead to increased lipid peroxidation, where free radicals ‘steal’ electrons from lipids in the eye, which results in cellular rigidity and deformability. This also induces oxidative stress that increases with age, where there is an imbalance between the production and accumulation of reactive oxygen species (ROS) in cells and tissues and the ability of one’s body to detoxify these reactive products [10].

Researchers have shown that the oxidative stress which is caused due to the accumulation of free radicals (atoms with an unpaired electron) plays a role in the pathogenesis/development of cataracts [3] and that this process can be prevented by antioxidants, which help stabilize the free radicals by donating a free electron, and metal carrier proteins, which bind to the metal ions and helps transport and store them [11].

Iron and Sulfate are commonly found in well water, as the Sulfur bacteria produce a slime and can help other bacteria grow, such as iron bacteria. The slime can clog wells, plumbing, and irrigation systems, resulting in unclean water.

1.3 Correlation of Transition Metals in Local Water Sources and Vision Impairment caused by Cataracts in Third World Countries

In many third-world countries, water is typically unregulated and comes from local sources of water, such as wells, nearby springs, etc. About 60% of water bodies in Ghana are polluted with most of them in critical condition (Ampomah 2017). [19] This water typically contains a higher concentration of transition metals. Asamoah-Boateng (2009) indicated clearly that arsenic, iron, and lead were the most predominant mining-related metallic pollutants found in all the water bodies [20]. River waters were moderately soft to slightly hard (91.20–111 mg/L CaCO₃ of hardness). High nutrient loads were observed in the basin and the study attributed it to agricultural, domestic, and industrial activities. There was reported total iron in the groundwater that ranged from 0.07 to 7.85 mg/L [1], whereas the average iron amount in US tap water is around 0.03 to 0.05 mg/L [5]. The presence of different ions such as carbonate (CO₃²⁻), phosphate (PO₄³⁻), sulfate (SO₄²⁻), and chlorine (Cl⁻) also affects the iron equilibrium in water. These ions have the potential to raise the water pH and effectively lower the photo-Fenton reaction rate. [6, 16] The general ionic dominance pattern of the water was Na > Ca > Mg > K, and HCO₃ > Cl > SO₄. The chloride dominance over sulfate was due to activities, fertilizer use,

household effluents, and other anthropogenic point sources [1] However, the combination of these factors still leads to the addition of H₂O₂ in enhancing both the photo-Fenton and TiO₂ photocatalysis reactions, which are present in the eyes. [6, 16]

Also, in these countries, the rate of vision impairment caused by cataracts is much higher compared to those in more developed countries. There is a high prevalence of visual impairment and blindness among the pensioners in Ghana, with the greatest cause being cataracts. [13]

Connecting previous ideas about oxidation causing cataracts, perhaps the high concentration of iron in the unclean water of developing countries could be a factor in increasing the rate and prevalence of cataracts forming. If this is true, it adds another reason why investing in better water treatment could improve health quality. The iron particles from naturally occurring build up, and without any sort of treatment, this iron will build up in the water, increasing the likelihood of developing cataracts. When this happens, especially in developing countries, access to treatment for cataracts is hard to find [2], despite only requiring a simple procedure, it can prevent one from any means of working and surviving.

2. Methods and Materials

2.1 Making the Solutions

To start, prepare the project materials. 1L of normal saline (0.9% NaCl saline), 3L of distilled water, 390 mg of ferrous sulfate, a measuring device, 8 beakers, and a stirring device were used to make the solutions. The normal saline will be used as a positive control. Next, a 0ppm of iron water solution, which is simply distilled water with no added ferrous sulfate, will be used to make the second solution. For the 130 ppm iron water solution, 65 mg of ferrous sulfate will be added to 500mL of distilled water. Mix until no particles are visible. For the 260 ppm iron water solution, 130 mg of ferrous sulfate will be added to 500mL of distilled water. Mix until no particles are visible. Repeat each batch of solutions. Ferrous sulfate is used here to help simulate some resistance to the Fenton reaction and to simulate water conditions in Ghana. Ferrous sulfate is also used as a common iron supplement for iron deficiency, so in addition to targeting oxidation caused by cataracts, it can also be used to test if intake of iron supplements may increase the development of cataracts. In total, there should be 4 solutions consisting of a positive control of normal saline, 0 ppm of iron water as a negative control, 130 ppm of iron water, and 260 ppm of iron solutions. Each batch made is for 1 pig eye. Use beakers to store and soak the pig eyes. It should result in 8 solutions for 8 eyes.

To maintain consistency, test the amount of iron with iron test strips. If the iron amount is off, adjust the concentration and test again until the desired concentration is met.

2.2 Soaking the Pig Eyes

Order the Pig Eyes last, and once the pig eyes are received, try and conduct the experiment as soon as possible once the pig eyes are used. Refrigerating the eyes may damage the proteins in the lenses once taken out of the packaging, and may lead to different/inaccurate results.

Place the eyes into the solutions of either normal saline, 0 ppm of iron water, 130 ppm of iron water, and 260 ppm of iron water. Make sure the eyes are facing up, with the iris/lens looking up. Seal the beakers with plastic/saran wrap to prevent external influences, and place them to rest in a closed tin foil interior environment for 24 hours. Set up a UV light above both at an equal distance of 12 cm from the pig eyes to initiate a Fenton reaction in the iron and let it build up reactive oxygen species in the eyes. The Fenton reaction takes around 90 minutes, so the 24 hours will give plenty of time for the reactive oxygen species to build up.

2.3 Dissecting the Pig Eyes for Data Collection

After 24 hours, take out the pig eyes. Discard the remaining solutions and place the pig eyes into a beaker with Carolina's Perfect Solution® or other similar solutions to help preserve the pig eyes while taking one and dissecting one at a time. Dissect the pig eyes with a dissection kit and dissect out the lenses. Reference demonstrations or other resources if necessary.

Measure the opaqueness of the lenses by shining a light through the lenses on top of a colored background of red, green, and blue, and a light screen. Take an image of the lenses with a digital camera. The RGB values and the hex values will be found from online tools (https://www.ginifab.com/feeds/pms/color_picker_from_image.php, <https://www.rgbtohex.net/>). From this, the color similarity can be found by comparing the hex values between the saline and the iron water-soaked pig lenses through another online tool (http://www.colortools.net/color_matcher.html) found as a percentage, as well as finding the luminance to quantify the data through an RGB to Luminance online tool (<https://contrastchecker.online/color-relative-luminance-calculator>). The amount of light shining through the lenses would be able to pass through will be quantified and recorded. Record results.

Conduct data analysis, doing an ANOVA t-test (one-way) to find if the results are statistically significant at $p < 0.5$ for the lens luminance. If the p-value is less than 0.5, then it can be concluded that there is a statistical difference between the lens' opacity/color with no condition compared to lenses soaked with different concentrations of iron.

3. Results

3.1 Data

After dissecting the lenses out and placing the lens on top of a light source, light screen, and colored paper of Red, Green, and Blue, pictured below are the results.

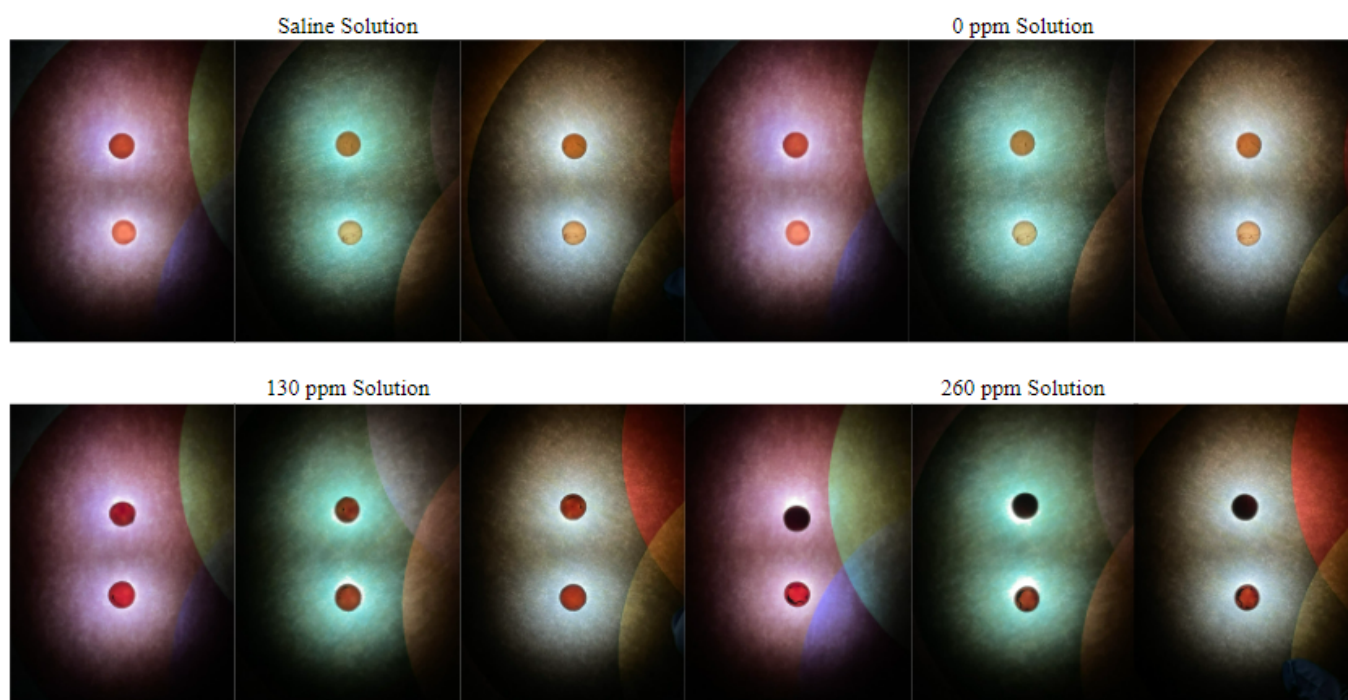


Figure 3. Image of Lenses. Lenses were taken out and put on top of a colored paper on top of a light screen and light. Photos were taken from an imaging device. These images were used for data collection.

From this collage of images, the RGB values and the hex values were found and put into a table. Top is referring to the lens on top in each individual picture, and Bottom is referring to the lens on the bottom in each individual picture. Online tools were used.

Using the Hex Values, I compared the control of normal saline soaked solution to the other ferrous sulfate solutions. An online tool was used to find the similarity.

	Red	Green	Blue
0 ppm Top	96%	100%	95%
0 ppm Bot	100%	99%	94%
130 ppm Top	79%	84%	88%
130 ppm Bot	77%	73%	74%
260 ppm Top	63%	63%	66%
260 ppm Bot	74%	65%	71%

Table 2: Comparison of color similarity of normal saline soaked lenses to other solution soaked lenses.

Using the RGB Values, I found the luminance of each lens. Luminance is the amount of light that can pass through it. This quantifies the color. An online tool was used to find the luminance.

	Red	Green	Blue
Saline Top	0.2207128151	0.2026908789	0.1844332171
Saline Bottom	0.3572303216	0.4452792548	0.3749742873
0 ppm Top	0.1416956892	0.2015884239	0.2014652646
0 ppm Bottom	0.341826463	0.4265480028	0.4413141712
130 ppm Top	0.0792715781	0.0705338997	0.0878123479
130 ppm Bottom	0.1354005089	0.1299551051	0.1136425719
260 ppm Top	0.0051570379	0.0067678874	0.0081971103
260 ppm Bottom	0.1109909403	0.0750605033	0.0892598999

Table 3: Color Relative Luminance

The Luminance's mean values were found for each color.

	Red	Green	Blue
Saline	0.288971568	0.32398506685	0.279703752
0 ppm	0.2417610761	0.314068213	0.321389718
130 ppm	0.107336044	0.100244502	0.060919841
260 ppm	0.060652508	0.040914195	0.048728505

Table 4: Luminance Mean Values

3.2 Statistical Significance

An ANOVA t-test (one-way) was conducted. The p-value is $< .00001$.

The result is statistically significant at $p < .05$ for the lens luminance.

Therefore, it can be concluded that there is a statistical difference between the lens' opacity/color with no condition compared to lenses soaked with different concentrations of iron.

	Treatments				
	1	2	3	4	Total
N	3	3	3	3	12
ΣX	1.7853	1.7544	0.537	0.3006	4.3773
Mean	0.2976	0.2924	0.0895	0.0501	0.182
ΣX^2	0.5334	0.5208	0.0506	0.0155	1.1202
Std.Dev	0.0209	0.0394	0.0224	0.0089	0.1183
Table 5: Anova t-test (one-way) results Luminance Mean Values					

4. Discussion

4.1 What These Results Mean

There is a statistically significant indicator that the presence of iron ions in drinkable water sources is a significant contributing factor to cataracts. From Table 2, there was approximately a 10-20 % change between the normal saline-soaked pig lenses and the 130 ppm soaked lenses. There was also approximately a 30-40% change between the normal saline-soaked pig lenses and the 260 ppm soaked lenses. This color difference is quite significant and shows how the increase in iron concentration in the iron caused more cloudiness to build up, signifying an increase in the rate of cataracts forming. This is further supported by the color relative luminance values, which showed a statistically significant difference between the saline-soaked lenses and the iron-soaked lenses, with the iron-soaked lenses having decreasing luminance as the ppm of iron increased.

These results show that iron in water is an inducer of cataracts. With the lens getting cloudier and allowing less light to pass through as the concentration of iron in water increases, it shows how more iron in water leads to a higher rate of cataracts. Also, since the lenses soaked in 0ppm of iron compared to the 130ppm and 260ppm of iron solutions has a dramatic color difference, it shows how the inclusion of iron does change the lens and makes them cloudier as cataracts, with more iron increasing the rate of cataracts forming.

The importance of these results shows another risk factor for unclean and unregulated water use. Water is not simply just distilled water and ferrous sulfate. There is much more that makes up water. If this one substance in unclean drinking water could lead to cataracts, on top of liver disease, heart problems, and diabetes, what about the other substances, such as the microbes or metals such as lead? I have simply listed one additional reason why reducing iron concentrations should happen already, and this experiment could help explain a factor that contributes to why cataracts are so prevalent in developing countries with poor water quality.

This information is useful for both local and national government agencies to look into finding ways to treat this high amount of iron. It notifies them of another issue that when solved, may help reduce cataracts. It is also useful to local people, as those who know they are drinking high concentrations of iron can understand the risks associated with doing so, and value the importance of clean water. They can start looking into known iron-reducing methods, such as using oxidizing agents and filters or chemically removing the iron to reduce the amount of iron consumed to a more reasonable amount. Other cheaper alternatives could be researched and looked into, as well as finding ways for better access to these treatments, especially for those in the developing world.

4.2 Comparison

This matches up with the idea of oxidation, oxidative stress, Fenton reactions, Photo-Fenton reactions, etc. This connects to published data and further establishes ideas of how Fenton reactions cause cataracts due to oxidative damage by showing that iron from drinking water can cause this whole sequence as well. The expected impact was not far off what was expected. Based on previous studies, by following the general concept of oxidation causing cataracts, this experiment followed the trend, and in my experiment, it connected the idea of iron in water causing oxidation, and oxidation causing cataracts.

4.3 Possible Errors

When experimenting, possible errors could have occurred, potentially lowering the confidence. One unexplained phenomenon was the difference between the lenses soaked in the same solution. Some of the lenses were considerably darker, which is visually evident in Figure 3. The two lenses in the same category had distinct color differences, with one being much darker than the other. Some possible reasons for this include that the UV light was not just causing the Fenton and Photo-Fenton reaction, and was causing something else to occur. Additionally, some iron had precipitated with the oxygen from

sulfate, leading to iron precipitation, which possibly could have been blocking certain UV lights. Human error when making the solutions is always a possibility, as there could be uneven concentrations made. The eyes being innately different could make this difference, as I have no way of verifying that the pigs were set in the same environment or have the same qualities.

Another problem that arose includes the fact that the lenses were hard, rather than soft as expected, so the data-gathering portion had to be adjusted and may not be the most accurate. The lenses being hard means that some of the protein already started denaturing, meaning some cataracts were already formed. However, this change could be adjusted and fixed as the positive control was found and could be used to correct for it, but also assumes that the eyes are from similar pigs.

Also, the measuring/data collection point could be different for each lens by a difference of a few pixels, which could lead to different results. Since the RGB values and hex values from the image were taken from only one pixel, the color values of the lenses may have shifted by a few pixels for each lens. After discussion with other reviewers of my project, I later learned that there is software that could take the same color values over an area rather than a single pixel, which could help increase the consistency of where the color is being taken from.

4.4 Additional Future Work

This research project shows the relationship of how iron in water can induce oxidation in the eye due to the Fenton Reaction, leading to cataracts. The results also allow for better understanding of what causes cataracts and shows how vital clean water is in developing countries. Furthermore, it further supports the idea of oxidative stress causing cataracts through Fenton reactions through water. This is another reason to look into better water treatment in developing countries. It can also provide the source of the problem to help reduce cataracts from forming.

From this, further steps can be taken to expand on this project, including further research. One factor that can be adjusted and looked at for more detail includes the concentration of iron ppm. Iron ppm used in this experiment was extreme, as normal water is around 0.3 to 0.5 ppm of iron at most in US tap water, and water in Ghana's water sources having no more than 10 ppm of iron. This experiment was to prove the concept of iron in water inducing cataracts, and further experiments could be done to find out if a minute amount of iron may lead to similar results, or if minute amounts of iron not significant enough to induce a similar outcome.

Iron is also not the only substance in water that makes up water sources in developing countries, such as lead and microbes, which have been looked at. Interactions between these substances in water could hint towards more issues that can be looked at. For example, the effect of excess iron could be reduced from Sulfur as stated in the introduction. The combinations of what makes up water can be analyzed to see if the initially observed effect for the individual material is induced more, induced less, prevented from happening, or even cause an entirely new effect. Besides forming cataracts, other health related diseases regarding iron could be analyzed and explored. In many developing countries, some other high rates of disease such as diarrhea, heart disease/cardiovascular disease, and liver cancer, to name a few. These could potentially be connected to the excess amounts of iron in drinking water used, and further work could be done to test it.

All of this would highlight the demand for iron filtration in water, and finding a way for developing countries to access cheap methods of cleaning water. It can help call attention to investing into proper water regulation, remodel the outdated equipment, and these same applications can be applied to wealthier areas as well.

5. Conclusion

5.1 Summary

These results support my hypothesis. We can tell the presence of iron ions in drinkable water sources is a significant contributing factor for cataracts because there is a statistically significant change in color and decrease in luminance in the iron water compared to the control. I concluded that the presence of iron in drinkable water sources was statistically significant with a p-value of $<.00001$ with approximately a 30% change in color. Iron in the water did undergo the Fenton reaction when powered by UV light and caused oxidation, damaging the lenses and forming cataracts.

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