

ANALYSIS OF FLOURIDE, CHLORIDE, CARBONATE, AND SULFATE IN FILTERED, TAP, AND GROUND WATER SAMPLES BY ISE AND TITRATION

Bunraj Grewal

ABSTRACT: This study aims to compare natural groundwater to tap water and filtered water in order to identify whether or not the groundwater has a significantly different concentration of FCCS from the household water. Consuming high concentrations of fluoride can lead to health issues such as fluorosis and reduction of IQ; both do not have effective treatment options, therefore, prevention of these health issues are necessary. Additionally, chloride, carbonate, and sulfate can make water less desirable to drink due to changes in the taste and texture. The concentration of fluoride, chloride, carbonate, and sulfate (FCCS) ions will be measured (with emphasis on fluoride due to its effect on human health) in tap water and filtered water for two households in the Pacific Northwest and will be compared to natural potable groundwater from the Artesian Well in Lynnwood. Fluoride and chloride will be measured using an ion-selective combination electrode, while sulfate and carbonate will be measured using the method of titration. The largest source of error will be from the interference of hydroxide when detecting concentrations of fluoride and chloride using the ion-selective combination electrode.

Fluoride ions are attracted to the positively charged calcium ions in teeth and bones. Too much fluoride can result in major dental, organ, and skeletal health issues such as skeletal fluorosis and kidney failure. The World Health Organization (WHO, 2008) set a maximum limit for the concentration of fluoride allowed in potable water at 1.5 ppm or mg/liter. Most of the fluoride intake gets stored in bone, dentin, enamel, and cementum (Gonzales et al., 2011). The cementum is a calcified bone-like substance that covers the root of a tooth. As one ages the concentration of fluoride in the cementum increases (Gonzales et al., 2011). Fluorosis, one of the most common effects of increased fluoride intake, causes mottling in teeth and is usually cosmetic, but could also result in severe enamel and cementum damage (Beltrán-Aguilar, Barker, & Dye, 2010). In the United States during 2004 the dental fluorosis was present in 40.6% of adolescents aged 12-15 and the prevalence was lower for adults at only 8.7% for ages 40-49 (Beltrán-Aguilar, Barker, & Dye, 2010). Human teeth are most vulnerable to fluorosis during the early ages of life when fluoride intake requirements are minimal and can be easily exceeded (Bottenberg, 2004).

Although potable water have a concentration of fluoride below 1.5 mg/liter, consuming anywhere around 1 mg/liter can increase the chances of fluorosis, especially when consumed in the first six years of life (Bottenberg, 2004). The treatment of skeletal and dental fluorosis is difficult and usually ineffective, therefore, preventing a high fluoride intake is necessary (Huber, Tobias, & Mosler, 2013).

This study aims to compare the fluoride, chloride, carbonate, and sulfate concentration in groundwater from the Artesian Well in Lynnwood, Washington to the fluoride, chloride, carbonate, and sulfate concentration of unfiltered tap water and water filtered through a refrigerator in order to determine if the groundwater is safe and beneficial to drink. Many residents in and around Lynnwood gather to fill gallons of water from that well and take it home to the rest of their family because the taste is preferred. This research applies specifically to that small population who consume untreated groundwater on a daily basis. Most potable water sources in the United States have enough fluoride, carbonate, chloride, and sulfate added into them to help prevent tooth decay and improve dental health, but the water in the community well is

untreated and could vary in concentrations of fluoride, carbonate, chloride, and sulfate. One study gathered over 60,000 measurements of fluoride concentrations in groundwater from 25 countries and created a global probability map using statistical modeling and concluded that there is a 20% probability that the groundwater in the Seattle area contains fluoride levels over the 1.5 ppm guideline from WHO (Amini et al., 2008, WHO, 2008). This research has implications of increased fluoride uptake for residents who drink untreated groundwater in the Artesian Well.

The concentrations of fluoride, chloride, sulfate, and carbonate in filtered water will be compared to the concentrations in tap water to determine if the water filters are actually reducing the concentration of the undesired chemicals. The Artesian Well, unlike household water supplies, contains continuous running groundwater that does not undergo a treatment process. Water samples from several different tap water sources (some from private in home sources others from community faucets and water fountains) will be examined for the concentration of fluoride, chloride, carbonate, and sulfate. The results will be compared to the levels in the Artesian Well to determine if there is a significant difference in concentration and whether it is beneficial to health and taste or whether it leaves citizens with too many undesired chemicals in their water.

Background

Exposure to fluoride in water has been associated with reduced performance, verbal, and full-scale IQ scores (Rocha-Amador et al., 2007). The effect of fluoride on IQ was tested by a study in China with children of age 10-12. After analyzing the data, results showed that children with high fluoride concentrations--over the 1.5 ppm--scored a lower average IQ (92.27) than the IQ of children with a low fluoride concentration (103.05). The difference between the groups was statistically significant,

therefore, the scientists concluded that fluoride can cause damage in brain function if too much is consumed from an early age (Lu et al., 2000). A more recent study concluded that there was an average decrease of 6 points in IQ in children who consume fluoridated water (Cheng & Lynn, 2013). Fluoride is also considered a neurotoxin linked to neurobehavioral disorders in children, and studies have shown that excessive intake cause central nervous system dysfunction (Shuhua et al., 2012; Yan et al., 2013).

Conventionally, fluoride was removed from contaminated water by liming and accompanying precipitation of fluoride. Various other methods used for the defluoridation of water are ion exchange, precipitation with iron(III), activated alumina, alum sludge, reverse osmosis, and electro coagulation. Adsorption is the most effective method and removes 80% - 94% of fluoride from an aqueous media such as water (Tomar & Kumar, 2013). However, removing too much fluoride in water is also problematic as it can lead to fluoride deficiency due to the fact that fluoride concentrations from other sources such as food are minimal, ranging from 0.02 mg/kg in milk to a maximum of 5 mg/kg in certain fish (WHO, 2008).

Bodies of water that are fluoride rich have been noted all over the world. One study collected groundwater data that involved concentrations of fluoride and created a predicted probability map of fluoride concentration exceeding the WHO guideline for drinking water (Amini et al., 2008). Based on this map, it is predicted that Africa and the Middle East have fluoride concentrations in their groundwater over the 1.5 ppm limit that is considered safe (Amini et al., 2008). Most fluoride in groundwater is not in ionic form. Fluoride-rich minerals like fluorite (CaF_2) and fluorapatite ($\text{Ca}_5\text{F}(\text{PO}_4)_3$), through the process of dissolution, are commonly the cause of fluoride ions being left over on rocks and soil that interact with water (Laurent & Marie, 2010). A recent study done in Brazzaville, Congo (Africa) showed that while the average concentration was 0.49 ppm of fluoride, some

areas had exceptionally high concentrations of fluoride, and the maximum concentration of fluoride in sampled groundwater was 2.90 ppm (Laurent & Marie, 2010). Another recent study done in Pakistan showed an average fluoride concentration of two different cities, Lahore and Sialkot, at 2.603 ppm and 2.819 ppm respectively (Tariq, 2014). These studies show that fluoridation in water is an international concern affecting many bodies of water across the world.

Water samples will also be tested for chloride, carbonate, and sulfate. Consumption of water with a high concentration of chloride can result in dehydration, and the presence of chlorine can affect the taste of water making it bitter. High levels of chloride are used in water treatment and not all of the chloride is removed afterwards which leads to a change in the taste. The WHO (2003) set a limit of chloride in drinking water to 250 ppm, which prevents dehydration and keeps the taste satisfactory. It was also determined by the WHO (1979) that chloride reacts with metal ions and creates soluble salts that increase the levels of metals in drinking water, which could be problematic for citizens drinking out of the Artesian Well (1979). Sulfate in drinking water has been known to cause short-term laxative effects with a sulfate concentration of 600 mg/L or more (Chien, Robertson, & Gerrard, 1968). There is no official concentration limit of sulfate in drinking water, although the WHO (2004) indicates that sulfate concentration over 500 mg/L causes a difference in taste. Carbonate in drinking water also affects the taste and whether the water is hard or soft. Hard water is created due to the excess presence of calcium and/or magnesium carbonate. The magnesium concentration is much lower than the calcium concentration in drinking water, which indicates that hardness due to calcium predominates. The concentration of calcium carbonate in potable water varies from 10 ppm to 500 ppm. The WHO (2003) has no limit on the amount of carbonate in drinking water because there is no conclusive evidence of a link between the

presence of carbonate and water hardness and negative health effects. Carbonate can also make water more corrosive resulting in the presence of heavy metals such as lead, copper, and zinc (Assembly of Life Sciences, 1977). These three chemicals are also biologically important and will be examined in this study.

Methodology

To measure the concentration of fluoride ions in the water, we will use a fluoride ion-selective electrode (ISE) from Thermo Fisher Scientific™. This procedure is based on a fluoride detection method using an ion-selective electrode published by the EPA (1974). The fluoride selective electrode produces an electric potential measured in volts, which can be related to the concentration of fluoride by using the Nernst equation. Lanthanum trifluoride (LaF_3), a solid inorganic surface, acts as the membrane of the electrode and attracts fluoride ions, but because the size of a hydroxide ion is similar to fluoride there is a possibility of interference if the solution is too basic. Fluoride ions pass through the membrane and cause a change in electrical potential inside the instrument by forcing the anions in the solution to move towards the membrane, which is then measured with respect to the reference. The fluoride ion-selective electrode contains an internal reference element, an internal reference electrolyte that will be 4 M KCl with AgCl, and the ion exchange crystal (Cheng, 2015; Bialkowski, n.d.). An external reference electrode is not necessary because there is an internal reference electrode included within the combination electrode.

We will collect tap water samples from the kitchen faucets of two different households in western Washington, and filtered water will be collected from the refrigerators of those exact same households after replacing the old refrigerator filters with similar brand new filters. Samples will also be collected from community water fountains and the Artesian Well in Lynnwood, which is a community water

source that runs using a reservoir of potable groundwater. Data from the well will be used to compare the results from the filtered and tap water sources to a source of natural untreated groundwater. Each sample will be collected by letting the water run for one minute and then placing similar one liter plastic (polyethylene) bottles into the flow to collect the running water. Each type of water sample will be collected five times and stored in one liter polyethylene bottles until analysis which could be up to four weeks. Since the tap, filtered, and ground water contains trace amounts of many different chemicals, the ion-selective electrode is a reliable method that can minimize interference easily.

Possible interferences could arise when determining the fluoride concentration if the pH of the sample is below 5 or above 10 due to fluoride ions bonding with hydrogen ions and also due to interference from OH⁻. However, the water samples collected should be just under neutral pH (Annual Water Quality Report, 2008). Other chemicals that may be present in water such as Cl⁻, Br⁻, I⁻, SO₄²⁻, HCO₃⁻, and PO₄³⁻ will not interfere with the electrode. The pH of the water being tested is predicted to be within 6-8 pH units, which indicates interference due to pH will be very minimal. The manufacturer has provided a detection limit of 0.02 ppm given that the solution in which fluoride ions are measured is neutral (User Guide Fluoride Ion-Selective Electrode, 2011). A study determining the fluoride concentration in the Antarctic krill successfully calculated the concentration of fluoride using this same ion-selective electron method at ~0.01 ppm, which is below the method detection limit given by the manufacturer (Lu et al., 2015). We expect our tap water samples to have a much higher concentration around 0.8 ppm due to a balance of limitations on the amount of fluoride allowed in drinking water and the addition of fluoride for oral health (Annual Water Quality Report, 2008). We predict that filtered refrigerator water would have a concentration of fluoride less than 0.8 ppm, assuming that the filter removes fluoride

ions, and we predict the groundwater from the Artesian Well is going to have a concentration of fluoride ions that is significantly greater than 0.8 ppm because it is unregulated.

The combination electrodes from Thermo Fisher Scientific™ will be blanked using deionized distilled water (DDW). Three standards will be created from a stock solution of 50 ppm sodium fluoride and will be diluted down to a range of 0.2-2 ppm based on the WHO (2008) limit of 1.5 ppm of fluoride in drinking water. To create the 0.2 ppm standard, we will pipette 1 mL of the stock solution into a 250 mL volumetric flask and dilute it using DDW. A standard of 1.1 ppm will be created by pipeting 2.2 mL of the stock solution into a 100 ml volumetric flask and diluting using DDW. We will create the 2 ppm standard by pipeting 4 mL of the stock solution into a 100 mL volumetric flask and diluting with DDW. All dilutions will be performed using a pipette with the range of 0.500 - 5.000 mL.

The concentration of chloride will also be determined in each water source using a chloride selective electrode that works similarly to the fluoride selective electrode. Chloride concentrations vary largely in drinking water sources, especially groundwater (WHO, 1979). To blank the chloride selective electrode, DDW will also be used. Four standards will be created from a stock solution of 500 ppm sodium chloride and will be diluted down to a range of 25 - 250 ppm based on the WHO (2003) suggested maximum limit of 250 ppm of chloride in drinking water. To create the 25 ppm standard, 5 mL of the stock solution will be pipetted into a 100 mL volumetric flask and diluted using DDW. A standard of 100 ppm will be created by pipeting 20 mL of the stock solution into a 100 mL volumetric flask and diluting using DDW. The 175 ppm standard will be created by pipeting 35 mL of stock solution into a 100 mL volumetric flask and diluting with DDW. We will create the 250 ppm standard by pipeting 50 mL of the stock solution into a 100 mL volumetric flask and diluting it with DDW.

Dilutions will be performed using a pipette ranging from 0.500 - 5.000 mL along with a 15 mL and 25 mL pipette.

We will generate a calibration curve using the results from the standards of fluoride and chloride by graphing the log of the standard concentrations (x-axis) against the millivolt values (y-axis) that come from the ISE. Concentrations will be calculated based on the Nernst equation. We will use a standard t-test to determine if the concentrations of the groundwater samples are significantly different from the filtered and tap water samples. T-tests will also be used to compare tap water with filtered water in order to determine whether filters actually remove a significant amount of fluoride and chloride. Using a t-test will provide a clear statistical analysis on whether the set of data collected from one potable water source is statistically significant from a different set of data collected from another source of water, therefore, a clear conclusion can be drawn with the data resulting in either a significant difference between chemical concentrations of the water sources or no significant difference observed. As shown in figure 1 below, t-test results will be displayed in tables, while concentration results will be displayed in a graph similar to those found in previous studies to make it easier to compare the collected data to previous data (Fojo, Figueira, & Almeida, 2013; Marrero, Torre, Fernández, Armendáriz, & Gironés, 2015).

To determine the concentration of carbonate we will titrate the water samples with 1 M hydrochloric acid along with 4 drops of bromcresol green indicator (Eng, 2001). To accurately determine the concentration of HCl used in the titration, we will titrate it with a known concentration of 1.000 M NaOH in triplicate. The HCl will be poured into the burette until the volume reaches the 50 mL mark for every reaction and the acid will be dispensed dropwise until the end point is reached. We will pipette 25 mL of the rainwater samples into a 200 mL beaker containing a magnetic stirrer and

the beaker will be placed on a hot plate with a magnetic stirring option. As the drops of HCl are added the stirrer will mix the sample with the acid readily until the solution turns into an intermediate green color after which the volume of HCl that was dispensed will be recorded. The results will be determined in molarity and will be converted to ppm afterwards. We expect the concentration of carbonate to range from 10 mg/L to 500 mg/L depending on whether the samples is ground water or filtered/ tap water; most water samples previously tested averaged around 100 mg/L of carbonate (Hardness in Drinking-Water, 2003).

Sulfate will also be analyzed using direct titration. We will titrate the water samples against a standard barium solution (Schroeder, n.d.). When using a barium solution, barium sulphate precipitates out. The method for determining sulfate concentration by titration that is going to be discussed next was developed by the Indian Standard Institution (Bhavan, Shah, & Marg, 1980). A barium perchlorate solution will be used for this experiment and is created by weighing 220 grams of barium carbonate and suspending it in water. Perchloric acid will be added to the solution drop wise until the carbon dioxide has bubbled out. The solution will then be heated gently on the hot plate while the carbon dioxide slowly boils out. After the solution cools it will be transferred into a 1 L volumetric flask, dissolved in 500 mL of water, and diluted to the mark with acetone. Acetone is used to speed up the titration time and helps the barium sulfate precipitate faster. The product will be a ~1 M barium perchlorate solution that we will standardize our 1 M barium perchlorate solution by titrating drop by drop against a known concentration of 0.100 M sulfuric acid. The 0.100 M sulfuric acid standard will be created by pipeting 10 mL of a 1 M stock sulfuric acid solution into a 100 mL volumetric flask and diluting to the mark with DDW. We will pipette 25 mL of our water samples into a 200 mL beaker containing a magnetic stirrer, place the beaker onto a hot plate with a magnetic

Average Fluoride Concentration

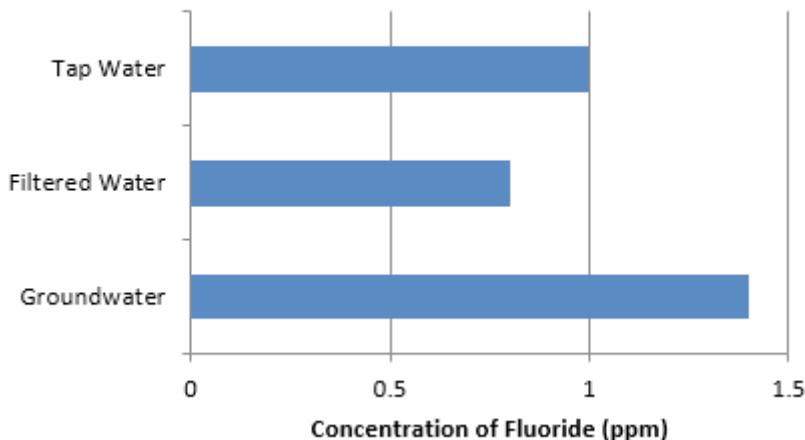


Figure 1: An example of a visual product of the data that will be collected based on graphs made by other scientists in past studies (Fojo, Figueira, & Almeida, 2013; Marrero et al., 2015).

stirring option, and titrate drop by drop against the barium perchlorate solution. The end point is detected by a blue-green color formed by sulphonazo III indicator. The results will be determined in molarity and will be converted to ppm afterwards. We expect the concentration of sulfate to be less than 250 mg/L for the tap water and filtered water, while the ground water could range anywhere between 400 mg/L -770 mg/L (WHO, 2004).

The concentration of carbonate and sulfate will be determined using the volume of the sample, the volume of the titrant was needed to reach the end point, and the concentration of the titrant used, which will be determined by the standardization step in each method. Results will be displayed in a table and as titration plots. A standard t-test will determine if the concentrations of the groundwater samples are significantly different from the filtered and tap water samples. Also to determine if filtered water removes a significant amount of sulphate and carbonate compared to tap water. A t-test is a reliable and accepted statistical examination for comparing two or more samples. The results from t-tests will clearly explain whether there is a significant difference between the chemical

concentrations calculated in the filtered water and the tap water and if there is a significant difference between the chemical concentrations calculated in the groundwater and the filtered and tap water.

See Appendix 1 for a list of materials that will be necessary for this experiment.

Communicating the Results

The scientists we will mostly be addressing will be dentists, but our paper will be available on the internet to all researchers. We plan to spread our information by educating dentists in local clinics about fluoride and other chemicals in drinking water so that they can address the topic with their patients appropriately. Dental clinics will be visited and a presentation will be given along with a print-out of the poster for dentists for reference. Most citizens that have fluorosis or are at risk of high fluoride intake will most likely visit their dentists, therefore, educating dentists can help them to advise their patients on the amount of fluoride they should intake and health choices they should avoid. Every dentist will also get a copy of the paper in case they wish to explore the topic in further

detail and choose to read through the references that were used in the paper.

For the general population, we plan to give demonstrations (in the form of hands-on activities and videos) of the connection between fluoride and dental health at local western Washington high schools. For our presentation, we will also find and refer to studies that are more applicable to the general population (such as fluoride in toothpaste), along with our study about fluoride in drinking water, due to their similarities. By adding more relative information and other causes of high fluoride intake into our high school outreach plan these presentations will better relate to non-scientists in the general public, and we can better help them understand the ubiquitous nature of fluoride. The study will be simplified and there will be a heavy focus on the results and the impacts to dental health that arise from an overabundance of fluoride in the system.

We will also set up a website or social media page (on Facebook or Twitter) and display both simplified and scientific information along with dentist information that could be easily found by the general populous or government officials. It is our goal to reach the population of citizens that could be affected by large intakes of fluoride and explain to them what they can do to reduce exposure and where they can find reliable information. If the groundwater samples at the Artesian Well clearly show a high concentration of fluoride, we will send (by mail and email) our paper and our poster to the state health department so they can evaluate our work and plan actions accordingly.

References

- Assembly of Life Sciences. Safe Drinking Water Committee. (1977). Drinking water and health. Washington, D.C.: National Academy of Sciences.
- Amini, M., Mueller, K., Abbaspour, K. C., Rosenberg, T., Afyuni, M., Moller, K. N., Sarr, M., & Johnson, C. A. (2008). Statistical Modeling of Global Geogenic Fluoride Contamination in Groundwaters. *Environmental Science & Technology*, 42(10), 3662-3668. Retrieved from Academic Search Complete [EBSCO] database.
- Annual Water Quality Report. (2008). Alderwood Water & Wastewater District 16, 173-210. Retrieved from http://www.alderwoodwater.com/site/content/documents/waterqualityreport_08.pdf
- Beltrán-Aguilar, E., Barker, L., & Dye, B. (2010). Prevalence and Severity of Dental Fluorosis in the United States, 1999–2004. *NCHS Data Brief*, 53, 1-7. Retrieved from <http://www.cdc.gov>
- Bhavan, M., Shah, B., & Marg, Z. (1980). Method for Determination of Sulphate (Volumetric). *Indian Standards*, 1-6. Retrieved from <http://www.law.resource.org>.
- Bialkowski, S. E. (n.d.). Measurement of Fluoride in Drinking and Natural Waters. Retrieved from <http://ion.chem.usu.edu/~sbialkow/Classes/565/fluoride.html>
- Bottenberg, P. (2004). Fluoride Content of Mineral Waters on the Belgian Market and a Case Report of Fluorosis Induced by Mineral Water Use. *European Journal of Pediatrics*, 626-27. Retrieved from Academic Search Premier [EBSCO] database.
- Cheng, H., & Lynn, R. (2013). The Adverse Effect of Fluoride on Children's Intelligence: A Systematic Review. *Mankind Quarterly*, 53, 306-347. Retrieved from <https://www.webofknowledge.com/>

- Cheng, K. L. (2015). Ion-Selective Electrode Calibration Solutions. Retrieved from <http://www.clarksonlab.com/vcWeir.pdf>
- Chien, L., Robertson, H., & Gerrard, J. (1968). Infantile Gastroenteritis Due to Water with High Sulfate Content. *Canadian Medical Association Journal*, 99, 102–104. Retrieved from Academic Search Complete [EBSCO] database.
- Eng, J (2001). Acid-Base Titrations: Determination of Carbonate by Titration with Hydrochloric Acid. Retrieved from <http://classes.uleth.ca/200103/chem24101/Expt3.pdf>
- Environmental Protection Agency (1974). Fluoride: Method 340.2. Cincinnati, OH: U.S. Environmental Protection Agency. Retrieved from http://www.caslab.com/EPA-Method-340_2/
- Fojo, C., Figueira, M., & Almeida, C. (2013). Fluoride Content of Soft Drinks, Nectars, Juices, Juice Drinks, Concentrates, Teas and Infusions Marketed in Portugal. *Food Additives & Contaminants: Part A*, 30(4), 705-12. Retrieved from <https://www.webofknowledge.com/>
- Gonzales, C., Hotokezaka, H., Karadeniz, E., Miyazaki, T., Kobayashi, E., Darendeliler, M., & Yoshida, N. (2011). Effects of Fluoride Intake on Orthodontic Tooth Movement and Orthodontically Induced Root Resorption. *American Journal of Orthodontics and Dentofacial Orthopedics*, 139, 196-205. Retrieved from Academic Search Premier [EBSCO] database.
- Huber, A. C., Tobias, R., & Mosler, H. (2013). Evidence-Based Tailoring of Behavior-Change Campaigns: Increasing Fluoride-Free Water Consumption in Rural Ethiopia with Persuasion. *Applied Psychology: Health and Well-Being*, 6(1), 96-118. Retrieved from Academic Search Complete [EBSCO] database.
- Laurent, M., & Marie, J. M. (2010). Groundwater Quality of Southeastern Brazzaville, Congo. *E-Journal of Chemistry*, 7(3), 861-69. Retrieved from Academic Search Complete [EBSCO] database.
- Lu, J., Qi, L., Guo, W., Song, Y., Jung, Y. A., Cheng, Y., & Jin, Y. (2015). Determination of Fluoride Concentration in Antarctic Krill (*Euphausia Superba*) Using Dielectric Spectroscopy. *Bulletin of the Korean Chemical Society*, 36(6), 1557-1562. Retrieved from <https://www.webofknowledge.com/>
- Lu, Y., Sun, Z. R., Wu, L., Wang, X., Lu, W., Liu, & S. S. (2000). Effect of High-Fluoride Water on Intelligence in Children. *Fluoride*, 33(2), 74-78. Retrieved from <http://www.fluoridealert.org/wp-content/uploads/lu-2000.pdf>
- Marrero, J., Torre, A., Fernández, A., Armendáriz, C., & Gironés, C. (2015). Evaluación Del Riesgo Tóxico Por La Presencia De Fluoruro En Aguas De Bebida Envasada Consumidas En Canarias. *Nutricion Hospitalaria*, 32(5), 2261-2268. Retrieved from <https://www.webofknowledge.com/>
- Rocha-Amador, D., Navarro, M. E., Carrizales, L., Morales, R., and Calderón, J. (2007). Decreased Intelligence in Children and Exposure to Fluoride and Arsenic in Drinking Water. *Cadernos De Saúde Pública*, 23, 579-587. Retrieved from Academic Search Complete [EBSCO] database.
- Schroeder, W. C. (n.d.). Direct Titration of Sulfate. *Industrial & Engineering Chemistry Analytical Edition*, 5(6), 403-406. Retrieved from <https://www.webofknowledge.com/>
- Shuhua, X., Liu, Z., Ling, Y., Fei, W., & Sun, G. (2012). A Role of Fluoride on Free Radical Generation and Oxidative Stress in BV-2 Microglia Cells. *Mediators of Inflammation*, 1-8. Retrieved from Academic Search Complete [EBSCO] database.
- Tariq, S. R. (2014). Multivariate Statistical Analyses of Fluoride and Other Physicochemical Parameters in Groundwater Samples of Two Megacities in Asia: Lahore and Sialkot. *Journal of Chemistry*, 1-11. Retrieved from Academic Search Premier [EBSCO] database.

Tomar, V., & Kumar, D. (2013). A Critical Study on Efficiency of Different Materials for Fluoride Removal from Aqueous Media. *Chemistry Central Journal*, 7(1), 51-64. Retrieved from Academic Search Complete [EBSCO] database.

User Guide Fluoride Ion-selective Electrode (2011). *Thermoscientific*. Retrieved from <http://www.thermoscientific.com>.

World Health Organization (1979). Sodium, Chlorides, and Conductivity in Drinking Water: A Report on a WHO Working Group. 1-62. Retrieved from <http://www.ircwash.org/resources/sodium-chlorides-and-conductivity-drinking-water-report-who-working-group-hague-1-5-may>

World Health Organization (2003). Chloride in Drinking-Water. *Guidelines for Drinking-Water Quality*, 2(2). Retrieved from http://www.who.int/water_sanitation_health/dwq/chloride.pdf

World Health Organization (2003). Hardness in Drinking-Water. *Guidelines for Drinking-Water Quality*. Retrieved from http://www.who.int/water_sanitation_health/dwq/chemicals/hardness.pdf

World Health Organization (2004). Sulfate in Drinking-Water. *Guidelines for Drinking-Water Quality*. Retrieved from http://www.who.int/water_sanitation_health/dwq/chemicals/sulfate.pdf

World Health Organization (2008). Guidelines for Drinking-water Quality - Volume 1: Recommendations. Retrieved from http://www.who.int/water_sanitation_health/dwq/fulltext.pdf

Yan, L., Liu, S., Wang, C., Wang, F., Song, Y., Yan, N., Xi, S., Liu, Z., & Sun, G. (2013). JNK and NADPH Oxidase Involved in Fluoride-Induced Oxidative Stress in BV-2 Microglia Cells. *Mediators of Inflammation*, 1-10. Retrieved from Academic Search Complete [EBSCO] database.

Appendix 1:

A list of supplies that will be necessary for this experiment.

Equipment / Materials	Size / Specifications	Quantity
Pipettes with tips	0.500 ml – 5.000 ml range 15 ml and 25 ml pipette	1 of each pipette, several boxes of tips
Thermo Scientific Orion pH/ISE meter	STAR A324	1
Volumetric flask	1 L and 250 ml and 100 ml	1, 2, & 2 respectively
Water	Deionized and Distilled	Copious
Beakers	50 ml, 100 ml, 200 ml, and 500 ml	3, 5, 2, and 1 respectively
Polyethylene containers w/ lids	1 liter	25
Thermo Fisher Orion Fluoride Combination Electrode	Model: 9609BNWP	1
Thermo Fisher Orion Chloride Combination Electrode	Model: 9617BNWP	1
50.00 ppm Sodium Fluoride	Pure stock solution	50 ml
500.0 ppm Sodium Chloride	Pure stock solution	250 ml
4 M KCl with AgCl	Fill solution	100 ml
1 M HCl Standard	Stock Solution	3 L
1.000 M NaOH Standard	Stock solution	500 ml
Hot plate with magnetic stirring option		1
Magnetic Stirrer	Small enough to fit into a 200 ml beaker and stir properly	1
Burette	Standard 50 ml burette	1
Bromcresol green	Indicator	100 ml
Barium Carbonate	Solid state	500 grams
2 M Perchloric acid	Stock Solution	100 ml
100 % Acetone solution		1.5 L
1 M Sulfuric acid	Stock solution	500 ml
Sulphonazo III	Indicator	100 ml

Appendix 2: Timeline

The experiment will be carried out over a course of ten weeks. Each week will have set goals to meet. The goals are:

Week	Goals	Calculations & Progress
1	Get instruments running - Calibrate and blank	- Test water samples from the lab sink to determine if the readings are reasonable
2	Run standards - Create working standards - Determine precision (R.S.D.) of I.S.E.	- Create calibration curve - Determine method detection limit
3	Collect samples - Label and store samples	- Write up sample descriptions and storage conditions (temp, cloudiness of water, particulate matter present, etc.)
4	Run samples - Create raw data tables	- Calculate averages, standard deviations, C.I. and R.S.D.
5	Run additional samples if needed & check for mistakes or outliers	- Gather additional data to improve S.D. & R.S.D. - Record outliers and apply statistical tests such as the Grubbs test
6	Analyze results - Determine relationships between water sources with statistical tests such as a t-test	- Create tables of concentrations - Run T-Tests and C.I. tests
7	Refine & interpret data - Create labeled graphs & titration plots - Provide strong statistical support for hypotheses	- Start writing the discussion section - Reject or accept hypotheses based on data
8	Get peer reviews from mentors and other scientists	- Finish writing paper
9	Create poster - Add visual appeal with a balance of text and white space	- Turn heavy scientific writing from the paper into more visual and focused scientific writing
10	Revise poster and paper for grammar, topic transitions, and organization	- Look at peer reviews & proof read paper and poster