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Cigarette litter leachates: a statistical study of elements in freshwater and saltwater

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A statistical study of elements in freshwater and saltwater

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Departmental Honors Thesis

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Contents

Abstract

Cigarette butts are the most common item found in coastal litter cleanups as approximately 4.5 trillion smoked cigarette butts are discarded into the environment every year. Cigarette butts can leach toxic heavy metals and nicotine along with other compounds from tobacco combustion. Past research in our lab has analyzed elements leached from cigarette butts into freshwater. The cigarette butts consisted of smoked and unsmoked butts that were soaked varying periods of time, and at different pH levels to identify the relationship of these variables to the amount of metal leached. The elements analyzed were Al, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sr, Ti, and Zn. The purpose of this research was to use additional saltwater data and to study the statistical differences in elemental leaching between the two types of water. The data was also compared to determine whether a significant difference exists in the elements leached from smoked and unsmoked cigarette butts. Since Al, Ni, Pb, and Ti were below the LOD in saltwater, no comparisons were made for these elements. It was determined that most comparisons between freshwater and saltwater were significantly different, with an exception of a few comparisons. For Mn, Sr, and Zn, all the comparisons were significantly different. However, for Cd there was only one significantly different comparison. Since the concentrations of the freshwater were generally higher than the saltwater concentrations this means cigarette butts have a higher impact on freshwater sources, affecting aquatic life. This study emphasizes the importance of addressing the cigarette butt litter issue and serves to supplement the lack of literature on the subject.

Introduction

Research Purpose and Objectives

The purpose of this research was to study the statistical differences in elemental leaching from cigarette butt litter between freshwater and saltwater matrices. Cigarette butts are one of the most littered items worldwide, ending up in oceans and freshwaters. The t-statistical test was used to identify whether any significant difference exists in the elements leached from smoked and unsmoked cigarette butts in the different matrices. The t-test was used for the comparison of the data as it can statistically evaluate differences between two experimental means, incorporating the standard deviations of the mean.[1] The program SPSS (Statistical Package for the Social Sciences) was utilized because it can process the large amounts of data which was collected for this study. This analysis links research of cigarette butts in saltwater with the freshwater data, both gathered by previous students. As literature on the subject of cigarette butt litter environmental impact is limited, this study can help fill the void on the subject.

Cigarette Litter

Cigarette butts are the most common item found in coastal litter cleanups as approximately 4.5 trillion butts are discarded into the environment every year.[2] Butt litter comprises up to 30% of the total litter on U.S shorelines, waterways, and land.[3] Due to the slow degradation, cigarette butts accumulate in vast quantities in the environment. A study by Thomson Patel found that 76.7% of smokers litter their cigarettes.[4] Much of the littering happens as soon as the smoker is done with the cigarette, throwing it directly on the ground. Some butts are stepped on or thrown in sewers to be extinguished. This littering problem is thought to be due to the ban on indoor smoking and the lack of ash receptacles in areas where smokers tend to smoke, such as at the entrances of buildings. Only 10% of cigarettes are disposed of in ash receptacles and people are more likely to litter butts if litter is already present. Their small size and plant material composition leads people to believe the butts are benign. However, it is estimated that governments and businesses spend $11.5 billion annually in litter cleanup efforts.[5] Once littered, butts are carried by the water cycle through different water reservoirs such as rain, rivers, and marine environments, where they can leach harmful substances.[1,6]

Some of the dangerous substances leached by butts include heavy metals. Cigarettes contain heavy metals because the tobacco plant uptakes elements from soils. Additionally, the tobacco leaves contain heavy metals because of chemical fertilizers, pesticides, and irrigation with residual water. Some heavy metals in tobacco may be higher concentration than others due to surrounding location, industrial / mining activities, and agronomic practices. The average heavy metal concentrations in cigarettes are shown in Table 1.[7] In addition to the metals in the tobacco due to the previously stated causes, 600-1400 additives are used in cigarette manufacturing, which may contain trace metals. In cigarette ash, 65-75 % of the metals are retained.[8] Cigarette butt litter risk is overlooked by the public as the negative effects on animal and human health is not notably visible, nor is there active education on the subject.

Table 1. Heavy Metals in Cigarettes	
Element	**Amount**
Al	699-1200 μg/g cigarette
Cd	0.5-1.5 μg/cigarette
Cr	0.002-0.5 μg/cigarette
Cu	156 μg/g cigarette
Pb	1.20 μg/cigarette
Mn	155-400 μg/g cigarette
Ni	0.078 μg-5μg /cigarette
Zn	24 μg/g cigarette

Environmental Impact

Oceans cover over two-thirds of Earth's surface. They play a vital role in global biogeochemical cycles and contribute a large part to the planet's biodiversity. Additionally, many people use the sea as a means of livelihood. However, marine debris has also emerged as a global conservation issue. Semi-synthetic bioplastic, also known as rayon**,** is widely reported in the marine environment. Environmental exposure causes the materials to degrade which results in micron-sized rayon fibers.[2]

Discarded cigarette butts usually consist of three components: unsmoked tobacco, filter, and wrapping paper.[9] Smoked cigarette butts are a notable source of rayon microfibers as each cigarette filter contains about 15,000 cellulose acetate rayon fibers. It is estimated that over 2 billion rayon fibers per square kilometer contaminate the sea bed. The acetate in cellulose makes the cellulose inaccessible to decomposing microbes. The slow degradation is also due to low nutrient content, especially nitrogen, which limits microbial activity.[3]

It is estimated that there are 7,000 chemicals released by tobacco smoke, with at least 70 of them being carcinogenic.[6] Some of these harmful chemicals are retained in the cigarette filter and leached into the aquatic environment when they are disposed. Cigarette butts can leach toxic heavy metals and nicotine along with other organic compounds from tobacco combustion such as hydrogen cyanide, ammonia, acetaldehyde, formaldehyde, benzene, phenols, and pyridines.[10]

Previous research has found cigarette butt leachates to be acutely toxic to fish, microbes, *cladocerans,* and insects.[3,10] A study of the impact of cigarette butts on the polychaete ragworm *Hediste diversicolor* found that that exposure to the cigarette butt leachate nicotine in seawater at a concentration of greater than or equal to 2 filters L^{-1} (172 µg L^{-1} nicotine) significantly inhibited burrowing behavior.[2] Greater concentrations led to reduced growth rates and increased DNA damage.[2] Additionally, butts can cause harm by posing a potential risk for ingestion by young children, domestic animals, and wildlife.[11] Domestic animals that consume cigarette butts have shown serious gastrointestinal, central nervous system, and cardiovascular signs of nicotine poisoning.[5] As mentioned previously, heavy metals are also leached from cigarette butts. Heavy metals are toxic to organisms and can accumulate over time, causing diseases or disorders even in low concentrations. In soil, heavy metals can have a negative impact on microflora. These metals cannot be chemically degraded and must be transformed into nontoxic compounds. Therefore, it is important to prevent cigarette butts from reaching the environment where they can leach these harmful substances.[12]

Statistical Analysis

Statistics is used to reliably predict what will happen in certain populations. A statistical study is performed on data collected to gain a better understanding of the population and test a hypothesis.[13] A statistical model is created to represent the collected data as closely as possible.[13] Fitting models that accurately reflect the data is important to understand whether a theory is true. Models are made of variables and parameters. Variables are elements that can differ across the samples. Parameters are measurable factors that define a system and determine its behavior. They are estimated from the data set such as the mean. The sample data is used to estimate the population parameters therefore, it is assumed that the mean of the samples is equal to the mean of the population.[13] Sample means can vary from the true mean and thus a confidence interval is calculated to describe the possibility that the boundaries set will contain the true mean.[14] The confidence interval is commonly calculated at 95% confidence level.[14] This means that the confidence interval covers the true value in 95 of 100 studies performed.[14] The smaller the confidence interval range, the more precise the data measurements.[14]

The t-test is a statistical test that determines whether the population means of two samples are significantly different from each other. The t-test is used when the sample size is small, and population standard deviation is unknown. The t-distribution is the distribution of a mean divided by an estimate of its standard error. The equation is shown in Equation 1,

$$t = \frac{\bar{x} - \mu}{s/\sqrt{n}} \qquad \text{(Equation 1)}$$

where $\bar{x}$ is the sample mean, μ is the population mean, s is the sample standard deviation, and n is the number of samples. A t- distribution has a lower peak and more area in the tails of the curve than a normal distribution curve (bell curve) due to its greater variability. The more the degrees of freedom increase, the more the t-distribution tends toward a normal distribution.

The null hypothesis (H_0) is a hypothesis that is trying to be disproven, rejected, or nullified with sample data. In this study, the null hypothesis states that the means are not statistically different between different water types: freshwater or saltwater, or between different types of cigarettes. The probability of the statement being true is tested. If this is rejected, the alternative hypothesis is accepted. The alternative hypothesis is an explanation of what is contemplated to be the cause of the phenomenon, which is the opposite of the null hypothesis. [15]

In the t-test, the null hypothesis is represented as H_0: $\mu(x) = \mu(y)$ and the alternative hypothesis is represented as H_1: $\mu(x) \neq \mu(y)$. In this, $\mu(x)$ and $\mu(y)$ represent the population means. It is said that at the 5% significance level (α, alpha), there is a 1 in 20 chance the null is rejected when it is true. In this study, the null hypothesis is that there is no significant difference in the mean concentrations leached from the cigarette butts into the saltwater and freshwater. The alternative hypothesis is that there is a significant difference. This means the probability of the alternative hypothesis is high.[15] If the absolute value of t is above a certain critical value, then the null hypothesis is rejected. The critical values are shown in a t-table. The degree of freedom of t-test is N1 + N2 – 2, in which N is equal to sample size.[13] The degrees of freedom is the number of independent deviations (x_i- $\bar{x}$) which are used in calculating the standard deviations that are free to vary. For this purpose, the degrees of freedom is n-2 because when n-2 are known, the last two deviations can be deduced.[15]

There are two types of t-tests: the independent and paired samples t-test. The independent t-test is used to compare groups that are not related in any way. For this study, the means represent the same variable (concentration), but for two different populations (saltwater and freshwater). The groups are independent from one another. For the paired samples t-test, the groups compared are related in some way, such as the samples being from the same individual, object, or related units. This can be collected data at different times from the same population. This study utilizes the independent samples t-test.[13,15]

A statistical model that tests a directional hypothesis is called a one-sided test. If using a significance level of 5% (0.05), a one-tailed test has all the α of 0.05 in one direction, testing the probability only in one direction. This gives more power to detect an effect by not testing the other direction. A model that tests a nondirectional hypothesis is a two-sided test.[13] If the significance level is 0.05, a two tailed test distributes 0.025 of probability on each tail. This tests for the possibility of the relationship in both directions. The study utilizes a two-sided test because a one-sided test is rare and ignores the difference between the groups in the negative direction.[13,15]

The ρ-value represents probability. It calculates the probability of finding a sampling outcome. It is used to determine whether the null hypothesis should be rejected or accepted. A small ρ-value indicates that the probability that the difference is pure chance is small. To conclude whether the null hypothesis or alternative hypothesis are true, significance limits are specified at a level of significance, α. The level of significance of 0.05 (or 5%) is the most common. If the ρ-value is less than 0.05, the result is significant, and the null hypothesis is rejected. Therefore, the alternative hypothesis is accepted.[14]

Past Research

Freshwater

Past research in our lab elementally analyzed leachates from cigarette butts in both freshwater and saltwater. The freshwater research was conducted by Jessica Moerman in Fall 2008 and Spring 2009. The focus was to determine the concentration (in mg element/kg of cigarette) of 12 heavy metals leached from smoked cigarette butts and whole unsmoked cigarettes in aqueous solutions. The elements analyzed were aluminum, barium, cadmium, chromium, copper, iron, manganese, nickel, lead, strontium, titanium, and zinc. Smoked cigarette butts were obtained from cigarette receptacles outside buildings on UTC's campus. Unsmoked cigarettes consisted of Marlboro, Camel, and Kool cigarettes, as these are the top selling brands in the United States.

Approximately 4.0 g of cigarette material was added to wide mouth HDPE bottles with 100 mL of deionized water adjusted to three different pHs.[16] The pH range of 4.0, 5.0, and 6.0 was chosen based on typical pH from rainfall and used to identify the relationship between pH and amount of metal leached.[16] In order to adjust to the desired pH, dilute sulfuric acid and/or ammonium hydroxide was added to Millipore water. For the smoked cigarette butt material, 2.0 g of filter and 2.0 g of tobacco/ash were added to each bottle. For the unsmoked cigarette butt material, four whole cigarettes: two Marlboro, one Camel, and one Kool were added to each container. The cigarettes were allowed to soak for 1, 7, and 34 days. Four sample sets were prepared for each soaking period. A sample set consisted of the three pHs in different bottles with either smoked cigarettes, unsmoked cigarettes, or blanks. After the soaking period, the samples were syringe-filtered with a 0.2 μm filter tip into the test tubes for analysis. Leachates were analyzed via inductively coupled plasma optical emission spectroscopy (ICP-OES) and compared to standards.[16]

Saltwater

The saltwater research was conducted by Myranda DeMailly in Spring 2016 and Summer 2016. The target elements were aluminum, barium, cadmium, chromium, copper, iron, manganese, nickel, lead, strontium, and zinc. Unsmoked and smoked cigarette butts were also used. Three different saltwater types were used: TopFin Aquarium Concentrate, Ricca Synthetic, and Carolina. The sample preparation was identical to freshwater. However, a gallium co-precipitation methodology was needed to extract the leached elements from the saltwater matrix. The analysis method (ICP-OES) could not process samples in a high salt content matrix due to the interferences they cause.[17] The salts deposit on the interface can lead to decrease in signal stability. In addition, the dissolved salts can cause spectral interferences.

Whole cigarettes were purchased for the unsmoked samples using the same brands as in the freshwater study. Used cigarettes were collected from covered ash trays outside of buildings on campus. Triplicates of each type of water and type of butt were prepared. The sample preparation began by measuring 100 mL of seawater into a wide-mouth HDPE bottle. Four whole unsmoked cigarettes were added to an HDPE bottle. For smoked samples, about 2 g of ash and about 2g of butts were used. The soaking period remained the same: 1 day, 7 days, and 34 days. After the soaking period, the samples underwent the co-precipitation of gallium process. Specifically, 40 mL of leachate solution was syringe filtered into centrifuge tubes. Then, 1 mL of 6 M NaOH was pipetted into the tubes to increase the pH. This was followed by the additional of

2 mL of Ga standard (1000 ppm) to assist in precipitation. The solutions were left to precipitate for two days.

After two days, the samples were centrifuged for 5 minutes at 4º C and 10,000 RCF (relative centrifugal force). The supernatant (containing the salt water matrix) was decanted. The precipitate was washed twice with 30 mL of pure water (HPLC grade). After a second washing, the solution was centrifuged again. The precipitate was then dissolved in 5 mL of trace metal HNO_3. Solutions were quantitatively transferred to 10 mL volumetric flasks, diluted with HPLC-grade water, and mixed. The final solution was syringe-filtered into test tubes, analyzed by ICP-OES, and compared to standards. The co-precipitation method resulted in a 4-times preconcentration and removed the salt matrix.

Experimental

SPSS: Saltwater Comparisons

The data from the saltwater and freshwater studies was statistically analyzed using t-tests with SPSS (Statistical Package for the Social Sciences). A t-test was used to compare the data as it can statistically evaluate differences between two experimental means, incorporating the standard deviations of the mean.[15]

First, the raw data from the ICP analysis was organized. Next, a process for organizing the data in SPSS was developed in order to correctly process the data. The variables identified were different cigarette type (unsmoked and smoked), water type, and soaking time. Once the variables were identified, the data was processed. T-tests were processed to determine if a significant difference exists in the elements leached from smoked and unsmoked cigarette butts as well as a comparison between the three different sources of seawater: Carolina, Ricca, and TopFin. Elements below the limit of detection (LOD) were not included.

Originally, all the elemental data was entered into the SPSS file and t-tests were attempted. However, the data has too many dependent variables for SPSS to process the information. The specific variables cannot be individually selected because SPSS only allows two variables for each test. Therefore, three separate files were created for each element: the water type variable, cigarette type, and number of days. This allowed for the t-tests to be run on the desired data. Independent samples t-tests were applied using two-tailed t-tests with concentration as the test variable. The independent t-test was used because it determines whether there is a statistically significant difference between the means in two unrelated groups. The grouping variable was selected based on the type of t-test. A t-test output for barium comparison of smoked freshwater to smoked saltwater 1 day is shown in Table 2.

Table 2. Sample SPSS t-test Output for Barium										
	Levene's Test for Equality of Variances		t-test for Equality of Means							
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	1.814	0.197	7.606	16	1.00 E-6	3.6708978	0.4826219	2.6477851	4.6940105
Equal variances not assumed			7.606	13.57	3.00 E-6	3.6708978	0.4826219	2.6326666	4.7091289

The independent t-test assumes the variances of the two groups being compared are equal. Variability refers to the spread of the data. It is a measurement of how data points diverge from the mean and how data points differ from each other. Variance is calculated by averaging the squared differences from the mean. SPSS determines if the two conditions, smoked and unsmoked, have nearly the same or different amounts of variability between scores. The result is shown as “Equal Variances Assumed” or “Equal Variances not assumed” under the Levene’s Test for Equality of Variances in the output. If the “Sig.” value shown is greater than 0.05, the null hypothesis stating there is no difference in variances between groups is accepted. Therefore, the first row SPSS output with “Equal Variances Assumed” should be used because the variability in the two conditions are not significantly different. If the “Sig.” value is below 0.05, the null hypothesis is rejected because the variability in the two conditions are significantly different. The second row SPSS output with “Equal Variances not Assumed” should be used because the variability in the two conditions is significantly different.

The significant difference was determined from the calculated ρ-value shown as “Sig. (2 tailed)”. If this value was greater than 0.05, the null hypothesis stating that the groups are not statistically different is accepted. If the value is less than 0.05, the null hypothesis is rejected which means there is a significant difference. To confirm, the t-value was also analyzed. A t-table was consulted to determine the critical value for a 95% confidence interval for a two-tailed test. The t-value is based on the degrees of freedom. For a sample size of 18, the degrees of freedom are 16. If the calculated t-value is bigger than the corresponding value from the t-table at 95% confidence interval, then this means the comparisons are significantly different. If the calculated t-value is smaller than the corresponding value from the t-table, then this means the comparisons are not significantly different.

After much consideration, it was determined that the t-tests between the different water types diluted the research and thus only the comparisons between all waters was kept. The comparisons are shown in Tables 3-10. 1D represents 1 day. **Unsm** represents unsmoked and **sm** represents smoked. Those comparisons that were found to be significantly different are marked with an asterisk, *.

Table 3. Barium Saltwater Comparisons

Comparison	df	ρ, r	t	Unsmoked (mg/kg)	Smoked (mg/kg)
1D unsm v. sm all*	16	1.00 E-6, NA	7.606	5.46	1.78
7D unsm v. sm all*	16	6.87 E-7, NA	7.868	8.84	4.38
34D unsm v. sm all*	11.695	3.00 E-6, NA	4.186	10.34	3.81

Table 4. Cadmium Saltwater Comparisons

Comparison	df	ρ, r	t	Unsmoked (mg/kg)	Smoked (mg/kg)
1D unsm v. sm all*	15	1.50 E-2, NA	2.833	0.12	0.10
7D unsm v. sm all*	16	6.00 E-3, NA	3.169	0.16	0.11
34D unsm v. sm all*	12	1.00 E-3, NA	4.186	0.10	0.03

Table 5. Chromium Saltwater Comparisons

Comparison	df	ρ, r	t	Unsmoked (mg/kg)	Smoked (mg/kg)
1D unsm v. sm all*	-	-	-	0.04	-
7D unsm v. sm all*	16	1.00 E-3, NA	3.875	0.16	0.01
34D unsm v. sm all*	16	5.00 E-6, NA	6.732	0.10	0.02

Table 6. Copper Saltwater Comparisons					
Comparison	**df**	**ρ, r**	**t**	**Unsmoked (mg/kg)**	**Smoked (mg/kg)**
1D unsm v. sm all*	8.086	2.00 E-3, NA	7.606	1.25	2.77
7D unsm v. sm all*	16	1.00 E-3, NA	4.013	0.80	1.83
34D unsm v. sm all*	16	3.09 E-1, NA	1.05	0.36	0.45

Table 7. Iron Saltwater Comparisons					
Comparison	**df**	**ρ, r**	**t**	**Unsmoked (mg/kg)**	**Smoked (mg/kg)**
1D unsm v. sm all*	8.138	3.31 E-4, NA	5.919	13.02	4.31
7D unsm v. sm all*	16	3.00 E-3, NA	3.558	17.19	9.39
34D unsm v. sm all*	16	1.00 E-3, NA	4.170	78.22	48.92

Table 8. Manganese Saltwater Comparisons					
Comparison	**df**	**ρ, r**	**t**	**Unsmoked (mg/kg)**	**Smoked (mg/kg)**
1D unsm v. sm all*	5.371	2.00E-6, NA	21.064	160.13	11.57
7D unsm v. sm all*	16	3.00E-6, NA	6.933	160.13	11.57
34D unsm v. sm all*	16	4.79 E-10, NA	13.258	35.62	19.16

Table 9. Strontium Saltwater Comparisons					
Comparison	**df**	**ρ, r**	**t**	**Unsmoked (mg/kg)**	**Smoked (mg/kg)**
1D unsm v. sm all*	13	5.00 E-6, NA	7.453	0.38	0.09
7D unsm v. sm all	16	3.47 E-1, 0.235	0.969	0.89	0.70
34D unsm v. sm all	16	9.80 E-2, 0.402	1.758	12.48	8.82

Table 10. Zinc Saltwater Comparisons					
Comparison	**df**	**ρ, r**	**t**	**Unsmoked (mg/kg)**	**Smoked (mg/kg)**
1D unsm v. sm all*	15	1.52 E-4, NA	5.022	5.32	3.63
7D unsm v. sm all*	16	4.79 E-4, NA	4.367	5.32	3.63
34D unsm v. sm all*	16	7.40E-8, NA	9.307	6.91	3.31

SPSS: Freshwater and Saltwater Comparisons

For the freshwater to saltwater comparisons, the data was grouped into SPSS files by element and day. For example, all the data for freshwater and saltwater that included 1-day barium leachate concentrations was grouped into one file. The results are shown in Tables 11-18. The determination of whether the values were significant or not were found in the same manner as the saltwater comparisons. The concentration comparisons were graphed as shown in Figures 1-16 at a 95% confidence interval.

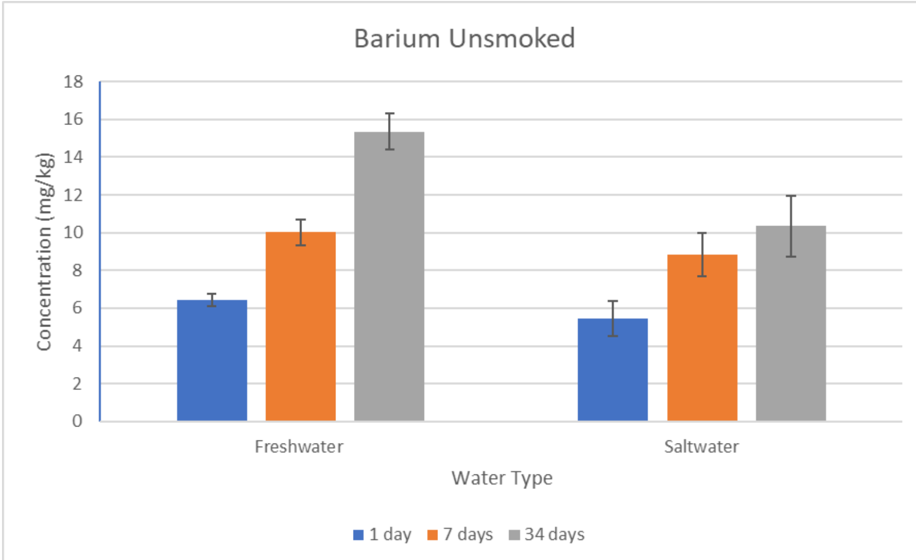

Figure 1. Barium Unsmoked

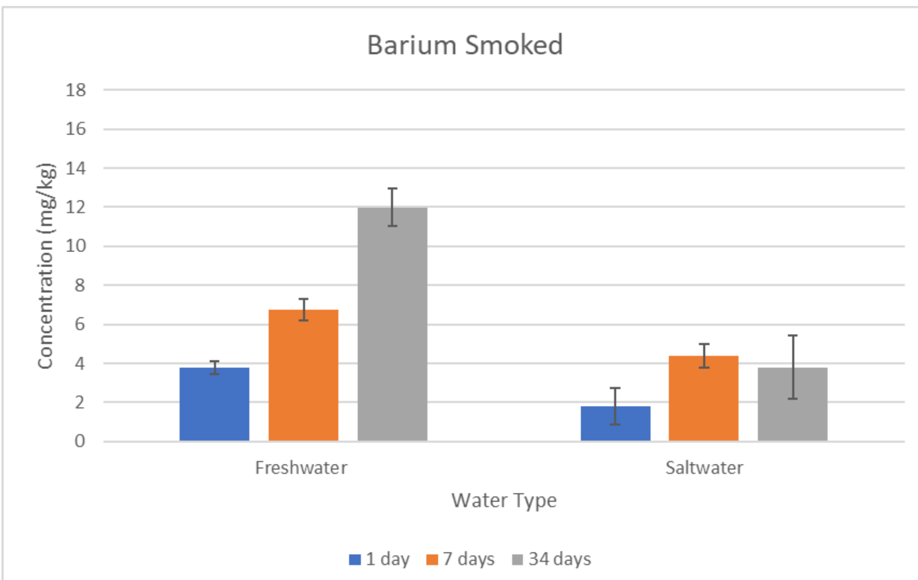

Figure 2. Barium Smoked

Table 11. Barium Comparisons

Comparison	df	ρ, r	t	Freshwater (mg/ kg)	Saltwater (mg/ kg)
1D sm fresh v. 1D sm salt*	8.821	3.70 E-5, NA	7.596	3.80	1.78
7D sm fresh v. 7D sm salt *	16	5.00 E-06, NA	6.705	6.70	4.69
34D sm fresh v. 34D sm salt *	9.005	3.30 E-05, NA	5.694	11.98	3.81
1D unsm fresh v. 1D unsm salt *	9.926	4.50 E-2, NA	2.294	6.44	5.46
7D unsm fresh v. 7 D unsm salt	17	5.60 E-2, 0.445	2.050	10.01	8.84
34D unsm fresh v. 34D unsm salt*	16	1.50 E-5, NA	6.103	15.34	10.34

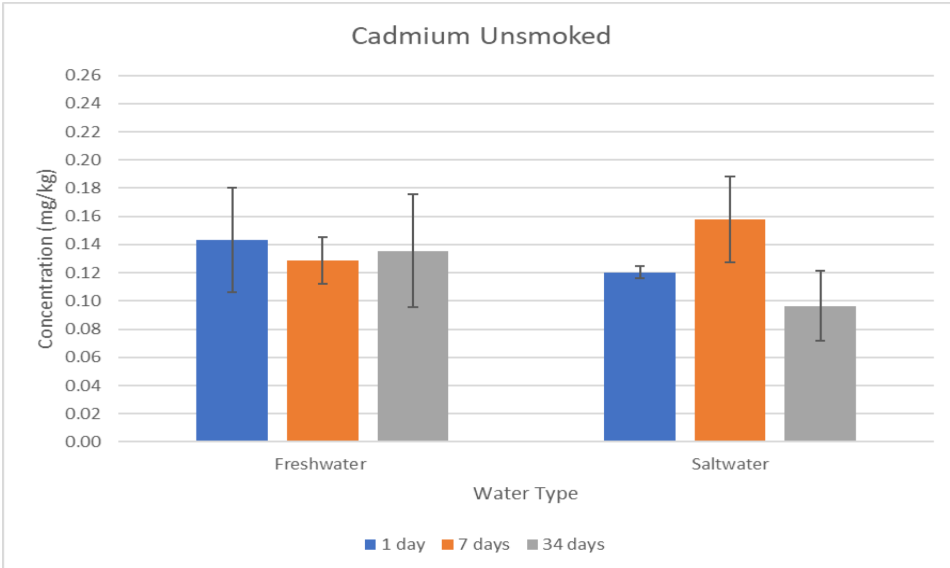

Figure 3. Cadmium Unsmoked

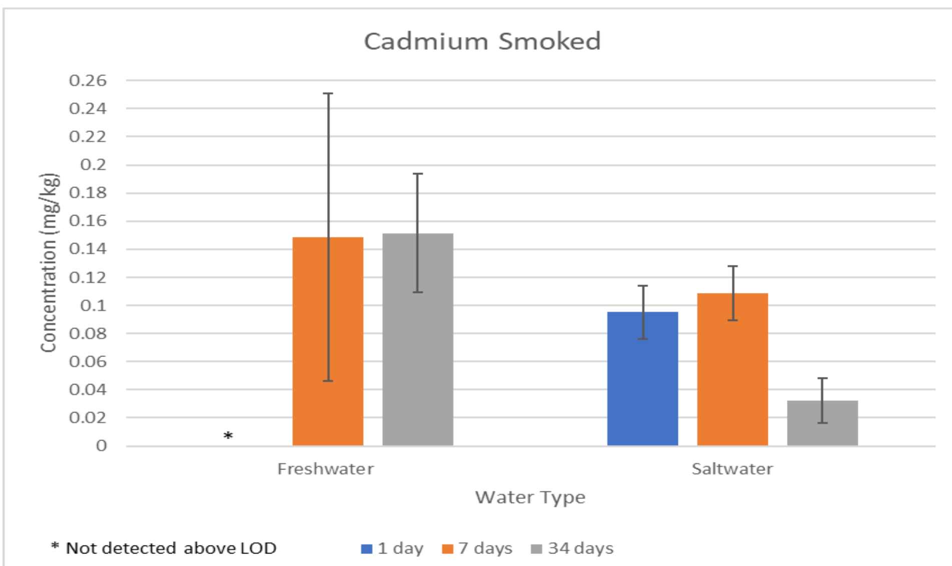

Figure 4. Cadmium Smoked

Table 12. Cadmium Comparisons					
Comparison	**df**	**ρ, r**	**t**	**Freshwater (mg/ kg)**	**Saltwater (mg/ kg)**
1D sm fresh v. 1D sm salt	8	-	-	-	0.10
7D sm fresh v. 7D sm salt	10	6.50 E-2, 0.548	2.072	0.15	0.11
34D sm fresh v. 34D sm salt *	8.385	1.79 E-4, NA	6.356	0.15	0.03
1D unsm fresh v. 1D unsm salt	7.203	1.89 E-1, 0.476	1.451	0.14	0.12
7D unsm fresh v. 7 D unsm salt	10.742	6.90 E-2, 0.525	2.021	0.13	0.16
34D unsm fresh v. 34D unsm salt	16	7.20 E-2, 0.433	1.923	0.14	0.10

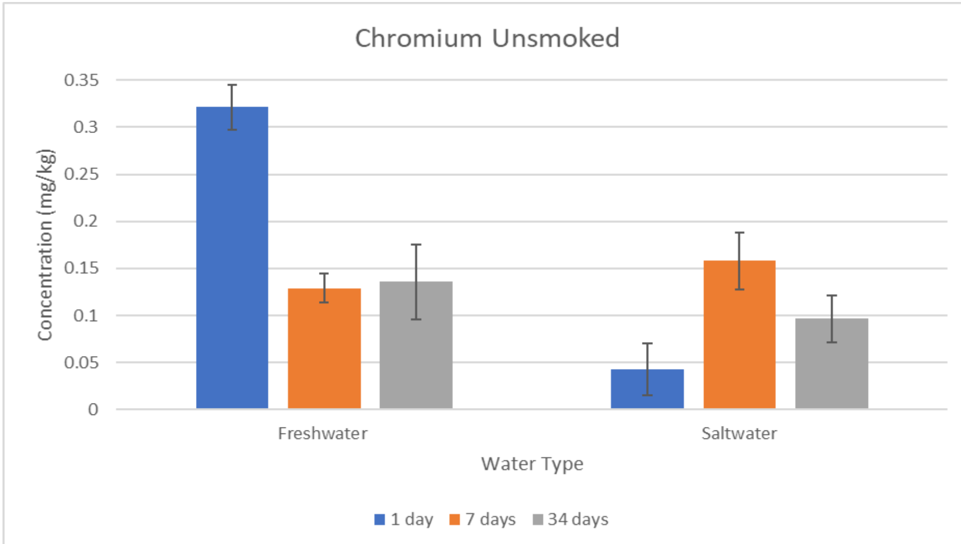

Figure 5. Chromium Unsmoked

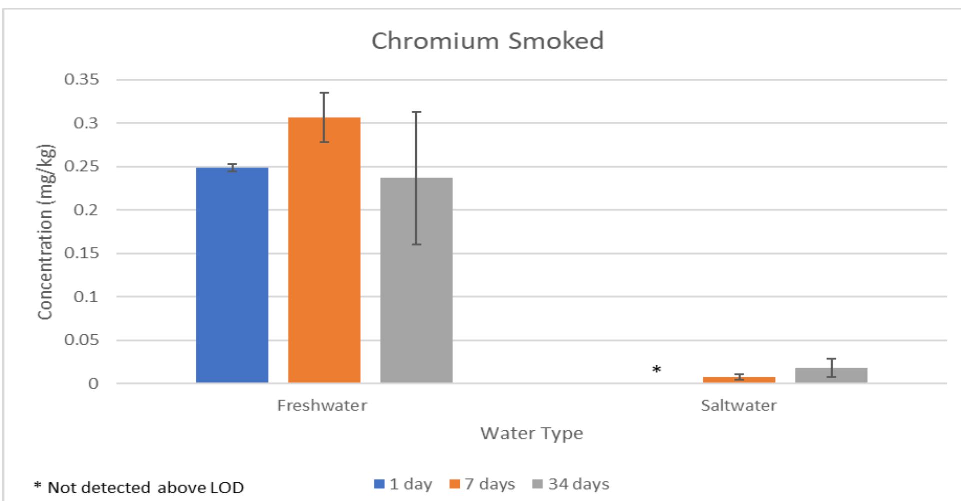

Figure 6. Chromium Smoked

Table 13. Chromium Comparisons					
Comparison	**df**	**ρ, r**	**t**	**Freshwater (mg/ kg)**	**Saltwater (mg/ kg)**
1D sm fresh v. 1D sm salt	8	-	-	0.25	-
7D sm fresh v. 7D sm salt *	9.224	1.60 E-9, NA	23.369	0.31	0.01
34D sm fresh v. 34D sm salt *	9.164	1.00 E-3, NA	4.859	0.24	0.02
1D unsm fresh v. 1D unsm salt *	13	1.32 E-10, NA	18.122	0.32	0.04
7D unsm fresh v. 7 D unsm salt*	16	1.09 E-22, NA	85.122	0.13	0.16
34D unsm fresh v. 34D unsm salt*	9.354	9.70 E-5, NA	6.469	0.14	0.10

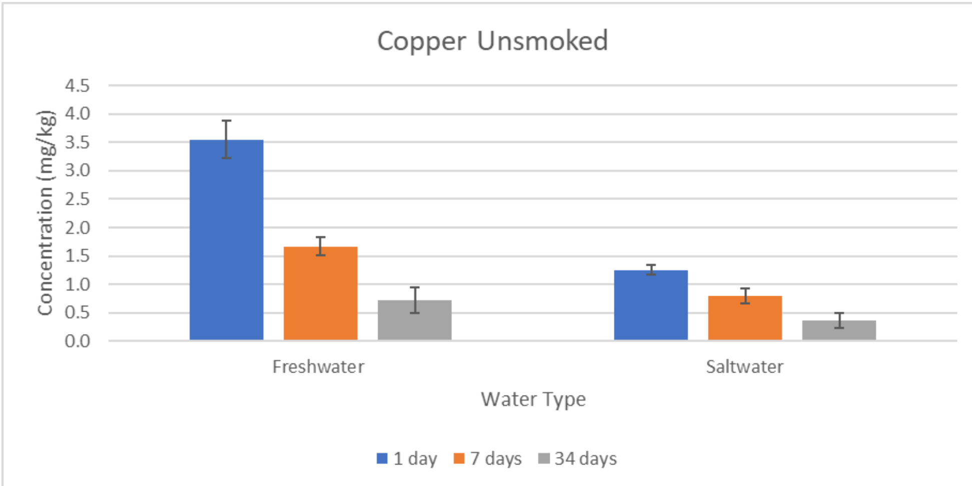

Figure 7. Copper Unsmoked

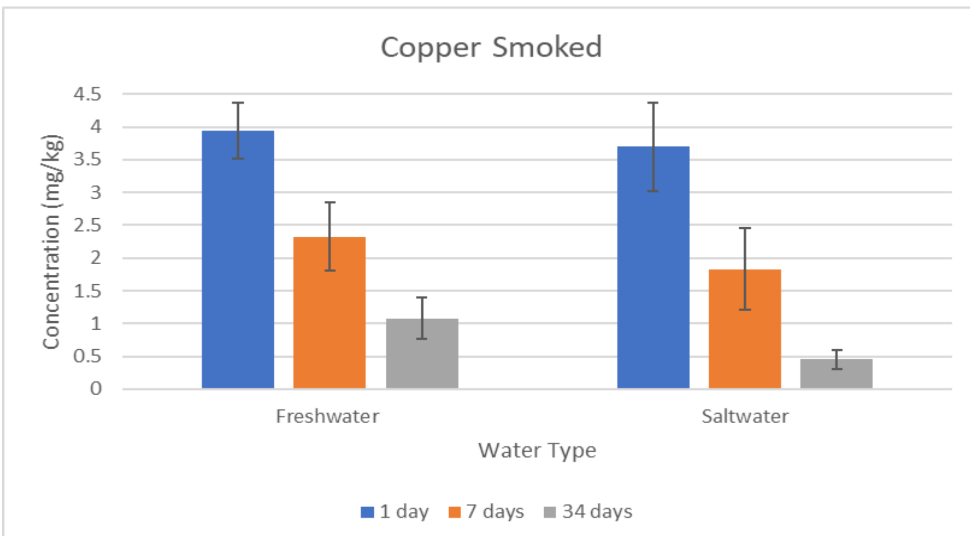

Figure 8. Copper Smoked

Table 14. Copper Comparisons

Comparison	df	ρ, r	t	Freshwater (mg/ kg)	Saltwater (mg/ kg)
1D sm fresh v. 1D sm salt	17	1.95 E-1, 0.311	1.349	3.94	2.77
7D sm fresh v. 7D sm salt	16	6.28 E-1, 0.123	0.494	2.33	1.83
34D sm fresh v. 34D sm salt *	14.929	1.00 E-3, NA	3.893	1.08	0.45
1D unsm fresh v. 1D unsm salt *	9.128	7.73 E-08, NA	15.387	3.55	1.25
7D unsm fresh v. 7 D unsm salt*	16	4.35 E-08, NA	9.674	1.67	0.80
34D unsm fresh v. 34D unsm salt*	17	7.00 E-3, NA	3.094	0.72	0.36

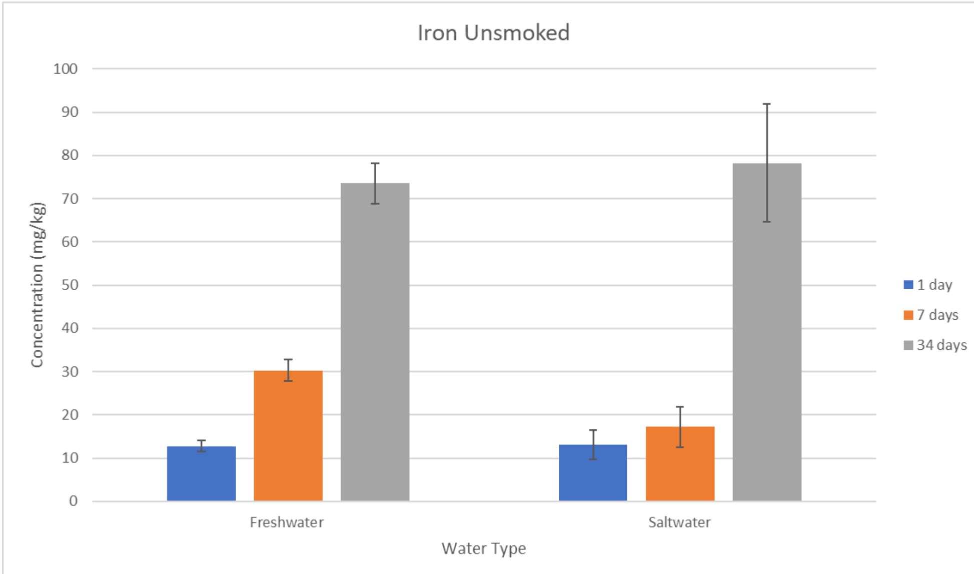

Figure 9. Iron Unsmoked

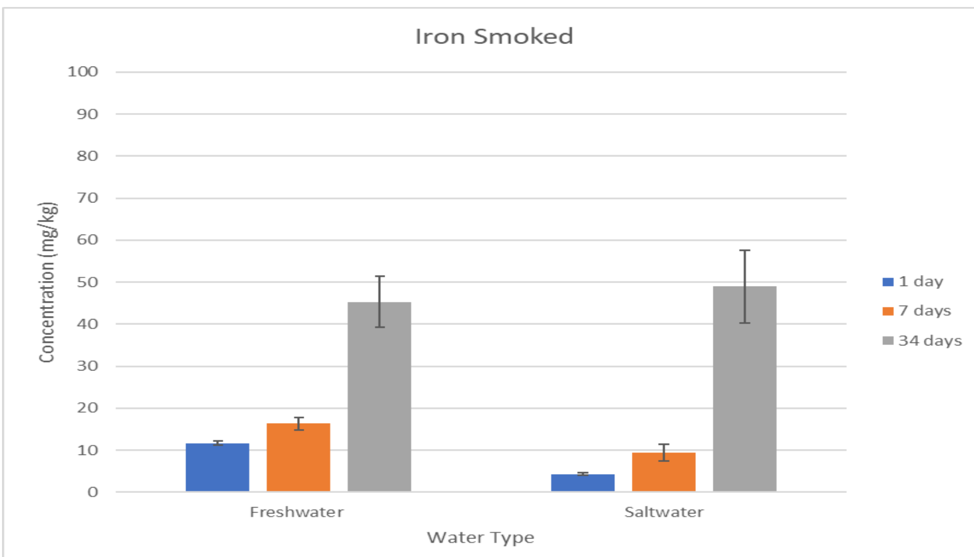

Figure 10. Iron Smoked

Table 15. Iron Comparisons

Comparison	df	ρ, r	t	Freshwater (mg/ kg)	Saltwater (mg/ kg)
1D sm fresh v. 1D sm salt*	17	2.68E-15, NA	26.619	12.77	13.02
7D sm fresh v. 7D sm salt *	16	9.00 E-6, NA	6.395	30.26	17.19
34D sm fresh v. 34D sm salt *	17	1.00 E-3, NA	0.792	73.56	78.22
1D unsm fresh v. 1D unsm salt *	10.254	8.78 E-1, NA	0.158	11.63	4.31
7D unsm fresh v. 7 D unsm salt*	16	3.30 E-5, NA	5.697	16.31	9.39
34D unsm fresh v. 34D unsm salt	9.873	4.74 E-1, 0.230	0.744	45.29	48.92

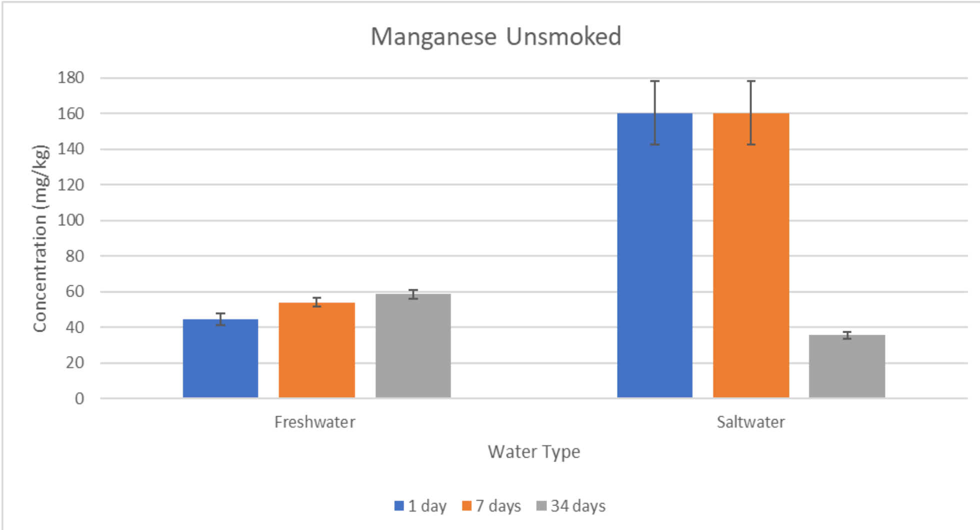

Figure 11. Manganese Unsmoked

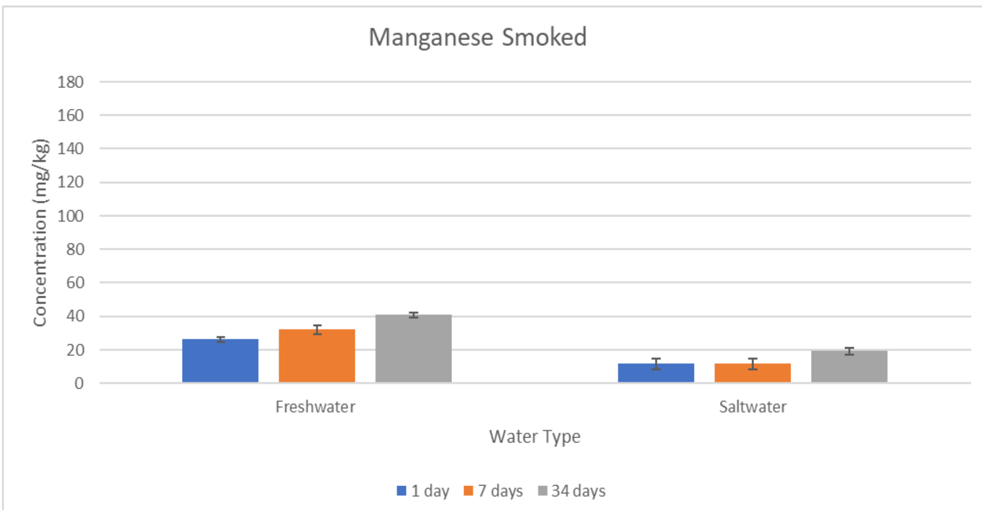

Figure 12. Manganese Smoked

Table 16. Manganese Comparisons

Comparison	df	ρ, r	t	Freshwater (mg/ kg)	Saltwater (mg/ kg)
1D sm fresh v. 1D sm salt*	7.463	1.60 E-5, NA	9.813	26.17	11.57
7D sm fresh v. 7D sm salt *	14	1.43 E-08, NA	11.608	32.12	11.57
34D sm fresh v. 34D sm salt *	17	1.14 E-12, NA	18.419	40.75	19.16
1D unsm fresh v. 1D unsm salt *	5.421	8.00 E-6, NA	16.4	44.20	160.13
7D unsm fresh v. 7 D unsm salt*	5.236	1.60 E-5, NA	15.163	53.89	160.13
34D unsm fresh v. 34D unsm salt*	16.459	9.15 E-12, NA	16.744	58.54	35.62

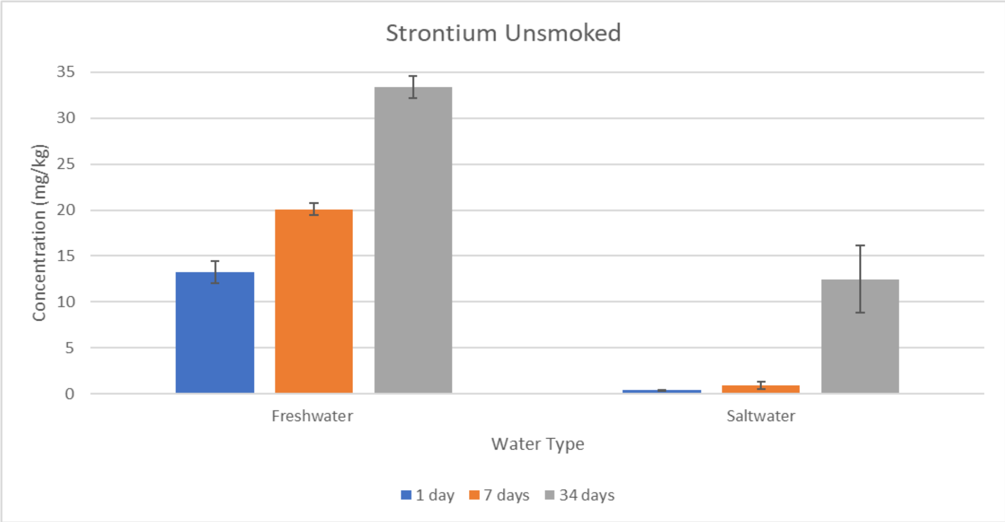

Figure 13. Strontium Unsmoked

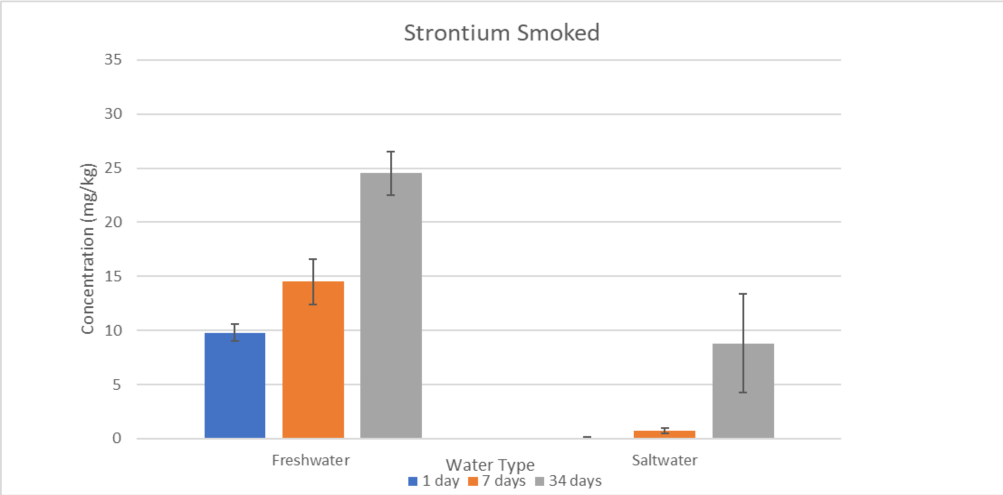

Figure 14. Strontium Smoked

Table 17. Strontium Comparisons

Comparison	df	ρ, r	t	Freshwater (mg/ kg)	Saltwater (mg/ kg)
1D sm fresh v. 1D sm salt*	8.039	2.38 E-09, NA	28.384	9.80	0.09
7D sm fresh v. 7D sm salt *	8.219	2.84 E-07, NA	15.06	14.52	0.70
34D sm fresh v. 34D sm salt *	16	2.00 E-6, NA	7.299	24.54	8.82
1D unsm fresh v. 1D unsm salt *	8.052	6.81 E-09, NA	24.807	13.23	0.38
7D unsm fresh v. 7 D unsm salt*	17	2.30 E-22, NA	69.983	20.09	0.89
34D unsm fresh v. 34D unsm salt*	8.542	1.59 E-4, NA	6.39	33.34	12.48

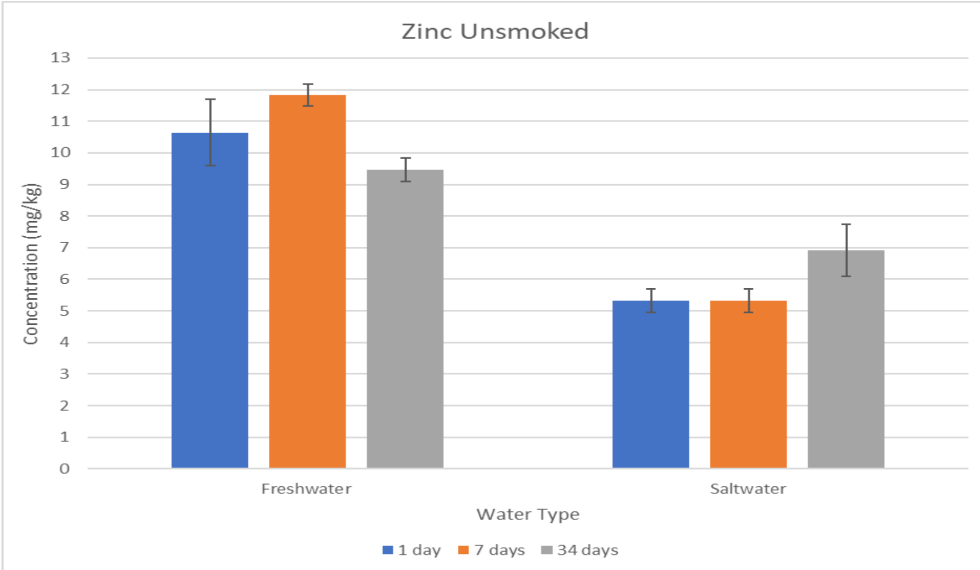

Figure 15. Zinc Unsmoked

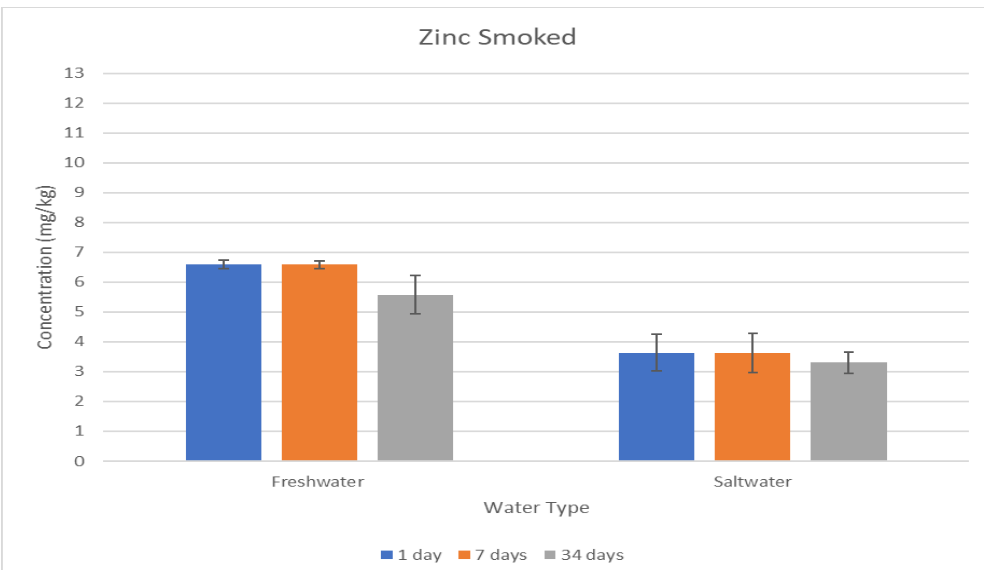

Figure 16. Zinc Smoked

Table 18. Strontium Comparisons

Comparison	df	ρ, r	t	Freshwater (mg/ kg)	Saltwater (mg/ kg)
1D sm fresh v. 1D sm salt*	9.156	7.00 E-3, NA	3.426	6.59	3.63
7D sm fresh v. 7D sm salt *	8.643	4.00 E-6, NA	10.207	6.59	3.63
34D sm fresh v. 34D sm salt *	16	2.00 E-6, NA	7.235	5.58	3.31
1D unsm fresh v. 1D unsm salt *	14	2.24 E-8, NA	11.206	10.64	5.32
7D unsm fresh v. 7 D unsm salt*	15	1.07 E-14, NA	29.501	11.82	5.32
34D unsm fresh v. 34D unsm salt*	16	7.00 E-6, NA	6.506	9.46	6.91

Discussion

Interpreting SPSS Output

The t-test is a statistical test that determines whether the population means of two samples are significantly different from each other. A sample t-test equation is shown in Equation 2 where $\bar{x}_i$ is the mean, s_i is the standard deviation, and n_i is the number of samples for each type, i.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \qquad \text{(Equation 2)}$$

The t-test assumes the data is normally distributed. The data from the freshwater and saltwater results were not tested for normality since the t-test is robust enough to assume normality.[18] This means that the t-test applies for non-normal data if the group sizes do not differ greatly. This is only true if the larger group is not 1.5 times greater in size than the smaller group. As our sample sizes are not vastly different, the t-test can be used.[19]

The adjusted values in "Equal Variances not Assumed" are a result of SPSS overcoming a violation to unequal variances. Unequal variances (heteroscedasticity) can affect the Type I error rate (underestimate the standard error) and lead to false positives.[20] The adjustment is to the degrees of freedom using the Welch-Satterthwaite method, which is robust enough to assume homogeneity of variance. This method calculates the standard error from the weighted average of the two variances instead of from the average of the standard deviation which is used when the variances are equal.[13]

The degrees of freedom for the "Equal Variances not Assumed" is significantly smaller in value than "Equal Variances Assumed" using the Welch adjustment. A smaller df increases the ρ-value. It may increase it past 0.05, which would mean there is no significant difference between the two groups. If it were not for the adjustment to the degrees of freedom, an incorrect assumption of significant difference may result.

For comparisons that had no significance difference, the effect value (r) was calculated to determine whether the effect is substantive. The effect value is shown in Tables 3-18. The effect size is calculated by Equation 3, where t is the value from the SPSS Output and df is the degrees of freedom. Table 19 show what the resulting r value means.

$$r = \sqrt{\frac{t^2}{t^2+df}} \qquad \text{(Equation 3)}$$

Table 19. Effect Size Meaning		
r value	**Effect size**	**Explains**
0.1	small	1% of variance
0.3	medium	9% of variance
0.5	large	25% of variance

The data was graphed with a 95% confidence interval. For samples with a t-distribution, the confidence interval (Equation 4) is used where x̄ is the mean, t_{n-1} is the t-value for the corresponding degrees of freedom, and SE is the standard error.

Confidence Interval = $\bar{x} \pm (t_{n-1} * SE)$ (Equation 4)

The standard error is calculated by Equation 5, where σ is the standard deviation and n is the number of samples.

Standard error of the mean = $\sigma/\sqrt{n}$ (Equation 5)

The standard deviation is calculated by Equation 6 where $\sum_i$ is equal to the sum of, x_i is the sample concentration, $\bar{x}$ is the mean of the concentrations, and n is the sample number.

Standard Deviation $= \sqrt{\sum_i (x_i - \bar{x})^2 / (n-1)}$ (Equation 6)

Saltwater Comparisons

The groups compared were smoked and unsmoked cigarette butts. It was found by Moerman that the pH did not contribute significantly to the leaching in freshwater.[16] Therefore, no pH studies were done with saltwater samples. For saltwater, Ni, Pb, and Al were not detected above the LOD and therefore no t-tests were run on this data. The element Ti was tested for saltwater, but the data was not analyzed due to difficulty detecting the emission peaks.

It was determined that all the comparisons for Ba, Cu, Fe, Mn, and Zn are significantly different. All the unsmoked concentrations are higher than the smoked for all these elements except Cu, whose smoked concentration is higher. Cd comparisons were only significant for 1 day comparisons. Cr 1 day smoked was not above the LOD. Therefore, no comparisons for 1 day were made. The rest of the comparisons were significantly different for Cr. Sr is only significantly different for 1 day comparisons. The concentrations for Sr, Ba, and Fe increase over time which is expected. The concentrations for Zn stay constant.

Typical concentrations of these elements found in seawater in previous studies are shown in Table 20. Over the time between the two published studies, the elemental seawater concentrations increased by factors of 10 to 100. Since the concentrations reported are in ppm (mg/L), the concentrations from the seawater experiments were also reported in ppm in Table 20. All the elements showed a higher concentration in the DeMailly study compared to the previous studies from 30 years ago and Australia's coastline seawater from 5 years ago. Although the concentrations from Australia's coastline are thought to be a result of wastewater, stormwater, and industrial effluents, the increasing number of cigarette litter worldwide can become another factor in heavy metal pollution in seawater. This heavy metal pollution has resulted in the deterioration of critical aquatic habitats such as seagrass and reef ecosystems.[21]

Table 20. Concentration of Elements in Seawater			
Element	**Average Concentration (ppm) Seawater 30 years ago[22-24]**	**Average Concentration (ppm) in Australia Seawater 5 years ago[21]**	**Average Concentration from UTC studies (ppm)**
Ba	3.0 E-3	-	4.80 E-1
Cd	1.1 E-4	8.0 E-4	4.04 E-3
Cr	5.0 E-5	4.7 E-4	3.25 E-3
Cu	3 E-3	2.7 E-3	2.1 E-1
Fe	1.0 E-2	2.5 E-1	3.44E -0
Mn	2.0 E-3	1.57 E-1	2.56 E-0
Sr	0.8 E-1	-	2.00 E-0
Zn	1.0 E-2	6.7 E-2	7.10 E-1

For the SPSS output, the t-value and ρ-value have an inverse relationship: the higher the t value, the lower the ρ-value. A higher t-value corresponds to a higher difference between the concentrations compared. The ρ-value is the probability of obtaining the observed results while assuming the null hypothesis (means are not significantly different) and the idea that the observed result is caused by random sample error is true. A lower ρ-value has a lower compatibility with these assumptions. That is why the null hypothesis is rejected for a small ρ-value. This means that for a 0.03 ρ-value, the probability that the observed data is due to random error is 3%. For all the comparisons that were significantly different, the ρ-value was very small meaning that the probability of the results being a consequence of random error is very small.[13,25] Since unsmoked concentrations were generally higher, this means unsmoked material (tobacco and wrapping paper) remaining on cigarette butts leach out higher concentrations of heavy metals in saltwater. This can be due to the formation of different organo-metallic compounds when the cigarette is smoked which may have different leaching behavior in saltwater or may be released in the smoke. More research is needed to confirm these assumptions. As many smokers do not smoke all of the tobacco to the filter before tossing it, this means that all of the cigarette butt is leaching heavy metals in saltwater, more so the unsmoked part.

It was observed that for Ba, Cd, Cu, Fe, and Mn the equal variances were not assumed for 1 day unsmoked to smoked comparisons for all waters. Equal variances were not assumed for the copper 7 day comparisons as well. This because the ρ-value from SPSS was not greater than 0.05 which accepts that the null hypothesis that the variability is not the same for the data sets. This means that the concentrations from one set varies much more than the concentrations in the other set. Therefore, the degrees of freedom were adjusted for these comparisons by SPSS using the Welch adjustment. This is thought to be due to the different initial leaching rates for unsmoked and smoked cigarette butts.

Freshwater and Saltwater Comparisons

There were three overall trends for the freshwater and saltwater measurements: increase in leaching over time, steady concentration over time, and decreasing concentration measurements over time. Elements are grouped into one of the three trending areas.

The increase concentration over time for both water types was expected and seen in Ba, Fe, Mn, Sr. These elements also had the highest concentrations leached compared to the rest of the elements analyzed. However, there are larger error bars on the saltwater samples compared to the freshwater. This is due to the additional processing needed to remove the saltwater matrix prior to ICP-OES analysis.

Zn and Cr showed a steady concentration over time meaning the elements leach quickly and then remained constant. There is little literature on heavy metal leaching into surface waters, but a study of heavy metal leaching from black tea leaves into water determined that Cr leaching was independent of brewing time, which agrees with the data.[26] Tea leaves are similar to the tobacco product since they are both leaves and could behave similarly during leaching. This could make them comparable.

As expected, concentration in unsmoked leachates is higher than smoked. This is thought to be from loss of heavy metal compounds during combustion. However, Cu and Cd showed a decrease in concentration over time. Leachate solutions have a pH of 5.5, which would prohibit precipitation of hydroxides. It is possible the Cu and Cd are attaching to the tobacco during the leaching period. If this is the case, the heavy metals are removed during the filtering step, before the ICP can analyze the concentration. Further research is needed.

Most comparisons are significantly different meaning heavy metals leach in different amounts in freshwater and saltwater. However, the leachates of metal-organics with heavy metal atoms are indistinguishable from meal ions because the ICP cannot identify the compounds, it can only detect the element. Therefore, the solubility and chemical reactions of the leachates of metal-organics cannot be studied and confirmed.

The comparisons which equal variances were not assumed are shown in Table 21. For these comparisons, the degrees of freedom was adjusted by the Welch adjustment by SPSS. These differences in variance can be attributed to the different leaching mechanisms for smoked and unsmoked cigarette butts in the different water types. However, more research is needed to confirm. As mentioned previously for the saltwater comparisons, the comparisons between freshwater and saltwater had very small ρ-values for those comparisons that were not significantly different meaning the probability of the data being a result of random error is low.

Table 21. Equal Variances not Assumed

Element	Comparison			
Ba	1D smoked	1D unsmoked	34D smoked	
Cd	1D unsmoked	34D smoked	34D unsmoked	
Cr	7D smoked	34 D smoked	34D unsmoked	
Cu	1D unsmoked	34D smoked		
Fe	1D unsmoked	34 D unsmoked		
Mn	1D smoked	1D unsmoked	7D unsmoked	
Sr	1D smoked	1D unsmoked	7D smoked	34D unsmoked
Zn	1D smoked	7D smoked		

Lower concentrations in saltwater were observed in most elements in comparison to freshwater. This is possibly due to the saltwater matrix affecting the leaching. The ion concentration in the saltwater matrix may shift the equilibrium towards the tobacco, limiting leaching into the saltwater. Since the freshwater samples were soaked in de-ionized water, the lack of ion concentration in the matrix may have encouraged greater leaching.

Most nonsignificant comparisons had a large effect size. Most effect values were around 0.4 which is close to the large effect size of 0.5 needed for a significant difference. This means the two values are far apart from each other enough for the difference to be substantial. The large confidence intervals are due to the small sample sizes. Specifically, the freshwater confidence interval for 7 day smoked is large due to a very small sample size of 3. Cadmium and chromium concentrations were determined to be below the limit of detection and are denoted by a star in the figures.

Since most comparisons were found to be significantly different and most freshwater concentrations are higher in concentration for most elements, it can be concluded that cigarette butts have a bigger impact on freshwater than saltwater. The concentrations obtained give an insight on the amount of heavy metals leached into freshwater sources in the environment. These heavy metals accumulate in organisms and can disrupt function in vital organs.[27,28] Certain organisms are more sensitive than others due to different factors such as age, sex, or size. In fish, the embryonic and larval stages are usually the most sensitive to pollutants.[29] Studies have shown that heavy metals are endocrine disruptors.[28] These disruptions can cause cancerous tumors, birth defects, and other developmental disorders.[30] Heavy metals can cause aquatic loss and an imbalanced food chain which disrupts the whole ecosystem.[31] These heavy metals make can travel through the food chain and affect humans that consume fish, especially in areas where the main source of food is fish.[31] Additionally, this contamination can affect groundwater and drinking water for humans.[27]

Conclusion

The purpose of this study was to determine if there is a significance difference in heavy metal leaching from cigarette butts in freshwater and saltwater. In addition, the significance was also calculated for cigarette butt leaching in saltwater for unsmoked versus smoked cigarette butts for different leaching time. The elements studied were Ba, Cr, Cd, Cu, Fe, Mn, Sr, and Zn. The data was obtained from past experiments performed at UTC. The researchers soaked smoked and unsmoked cigarette butts in freshwater and saltwater for 1, 7, and 34 days. Afterwards, the samples were analyzed by ICP-OES. The saltwater experiments used TopFin, Ricca, and Carolina seawater. The saltwater matrix was removed using a gallium co-precipitate method. The freshwater experiments used de-ionized water for the soaking. For the statistical comparisons, the SPSS program was used to run the t-tests because of its ability to process large amounts of data. The t-test was used because it analyzes the different means, is robust enough to assume a normal distribution, and is used for small sample sizes. The SPSS program was also used because it can adjust the degrees of freedom using the Welch formula if the variances between the two data sets compared are not equal, avoiding a Type I error. The significance was determined by the p-value given by the SPSS output. If the ρ-value was below 0.05, the null was rejected, and the comparisons was significantly different.

For the saltwater comparisons, all were found to be significantly different when comparing smoked and unsmoked leachates. Since unsmoked concentrations were generally higher, this means unsmoked material remaining on cigarette butts leach out higher concentrations of heavy metals in saltwater. This is thought to be due to the formation of different organo-metallic compounds in the smoked cigarette which may have different leaching behavior or are lost in the smoke. It was determined that most of the comparisons were significantly different for the freshwater and saltwater comparisons. The r-value calculated for those comparisons not significantly different was around 0.4, meaning it had a large effect. Since the concentrations were generally higher in freshwater, this means the freshwater sources are being affected more than saltwater sources. This can in turn affect development of aquatic life by bioaccumulation of heavy metals in their system which can disrupt the ecosystem as well as cause physical harm if the butts are ingested. Additionally, this contamination of freshwater sources can affect drinking water for animals and humans. This study emphasizes the negative effects of cigarette butt littering and is an addition to literature on cigarette butt leaching.

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