Statistical Consultant Project

Stat 496

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Introduction

This biological experiment was conducted in order to analyze the effect that different salts, often used as de-icing agents on public roads, have on plant growth. In particular the two salt compounds being studied were sodium chloride (NaCl) and calcium chloride ($CaCl_2$). These two salts were chosen as they are used by the city of Edmonton on sidewalks, roads and bike paths to aid in the melting of ice. The researchers hypothesized that $CaCl_2$ would have a less harmful effect on plant growth due to potentially positive effects of the calcium ion. This hypothesis is supported by research indicating that calcium ions "could effectively stimulate growth and alleviate deleterious impacts of water deficit" (Kang, et al., 2017). If $CaCl_2$ is found to be less detrimental to plant growth it would be beneficial for the environment for the city of Edmonton to use $CaCl_2$ as its primary de-icer.

Methods

Experiment Design

The first experiment studied the effect of the different salts on root length, root diameter and root hair presence. Five treatments were considered: control with no salt, low level of NaCl, high level of NaCl, low level of $CaCl_2$ and high level of $CaCl_2$. Thirty petri dishes with seven seeds each were equally divided among the five treatments. Each day for four days, a seedling was randomly selected from each dish and examined. Measurements taken included the length of the root, the diameter of the root at 0.5mm and at 1mm of growth. It was also observed if the root had developed root hair or not. After being examined the seedling was discarded as its growth would be stunted by human interaction.

The second experiment conducted looked into the effect of the salts on plant germination rate and true leaf development. The treatments were the same five as previously mentioned with the addition of a sixth treatment composed of a combination of both salts at the low levels. This experiment was conducted on 180 pots with five plants each equally split among the six treatments. The experiment setup is visualized in Figure 1. Over the course of approximately three weeks, the pots were periodically observed, and it was recorded how many seedlings, if any, out of five had germinated. On two of these dates the proportion of seedlings that showed leaf development from those that had germinated was also recorded.

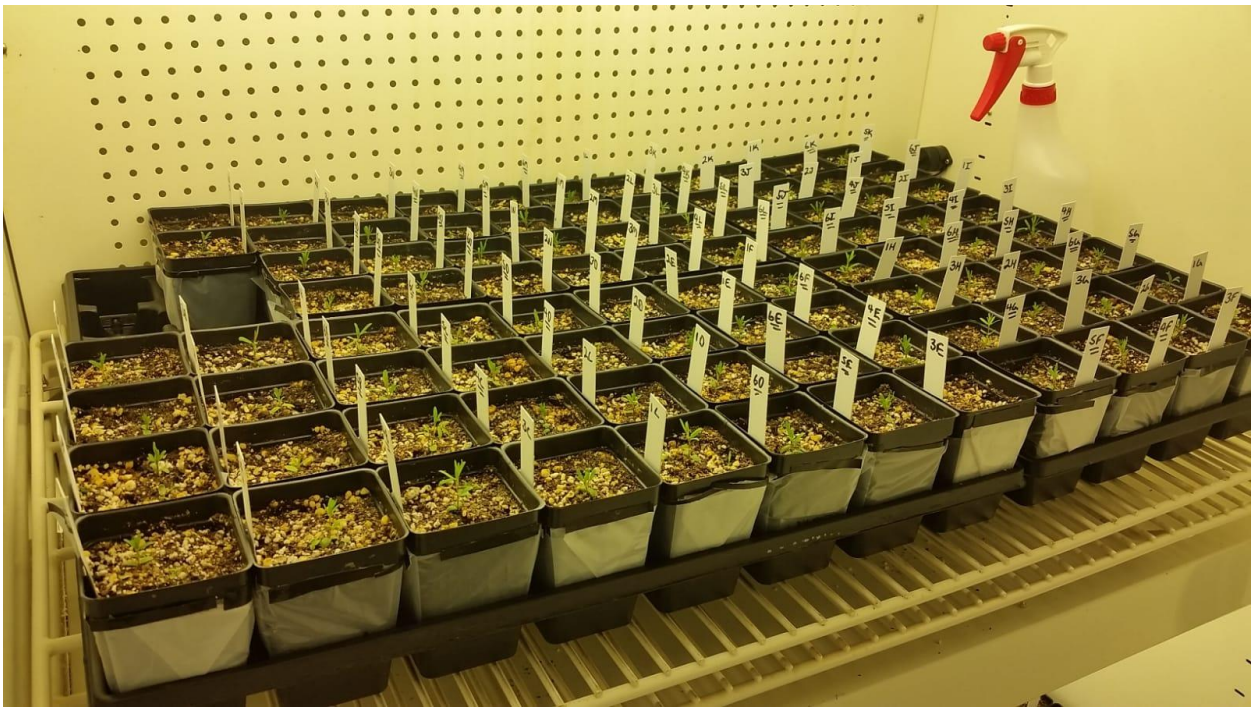

Figure 1. Experimental setup of germination and leaf development experiment.

Significance Level

A significance level of 5% was used throughout the analysis. When multiple comparisons were conducted, a Bonferroni correction was applied.

Sample Size

Presence of Root Hair, Root Length and Diameter

Data were gathered from an experiment performed on thirty petri dishes each containing seven seeds. Among the thirty dishes, six were assigned to each of the five treatments. Each day one seedling from each dish was selected and measured.

Germination Rate and True Leaf Development

This experiment was performed on 180 pots, with five seedlings in each pot. These pots were equally split among the six treatments allowing for thirty pots per treatment. Each day all of seedlings in all of the pots were observed.

Statistical Analysis

Data were analyzed using SPSS (version 26) on Mac. As no root hair was seen in the first day, and only three plants saw root hair in days two and three combined, these three days were collapsed into a before day 4 variable. A table of counts as well as a bar plot was created as

descriptive statistics. Association between treatment groups and root hair presence at various days was analyzed using Fisher's exact test followed by post-hoc multiple comparisons to test which treatments differ at an adjusted significance level of 0.5%.

The root length and diameter data were analyzed via repeated measure ANOVA. In the experiment design, the same root was not able to be measured at each day as the root needed to be removed in order to be measured and discarded following removal. However, repeated measure ANOVA is appropriate as daily measurements were taken from roots belonging to the same petri dish and therefore under identical conditions. Three separate repeated measure ANOVA models were fit with the different response variables being root length, root diameter at 0.5mm of growth and root diameter at 1mm of growth. Multiple comparisons were found in order to determine which treatments and which days differ in their effect on root length and diameter. A residual analysis found six observations that may be outliers for the root length model. Similarly, two potential outliers were found for diameter at 0.5mm and five for diameter at 1mm. After it was confirmed that these observations were properly recorded it was concluded that they would not be excluded from the analysis as they were true measurements.

Due to the binary nature of the germination data (1 = germinated, 0 = not) logistic regression was used to analyze it. A logistic regression model with germination as the response was fit to the data. The predictor variables were treatment, day and the interaction between treatment and day. Multiple comparisons were found in order to determine which treatments differ in their effect on germination. True leaf development was analyzed by viewing descriptive statistics as most of the treatments showed no leaf development.

Results

Root Hair First Occurrence

Descriptive Statistics

As seen in both Table 1 and Figure 2, the treatments involving NaCl were responsible for the only observations without root hair with five and six occurrences attributed to the low and high levels of NaCl respectively.

Table 1. First occurrence of root hair counts in each treatment group.

Treatment	**First Occurrence of Root Hair Counts**		
	Before Day 4	Day 4	Never
1 (Control)	2	4	0
2 (Low NaCl)	0	1	5
3 (High NaCl)	0	0	6
4 (Low $CaCl_2$)	0	6	0
5 (High $CaCl_2$)	1	5	0

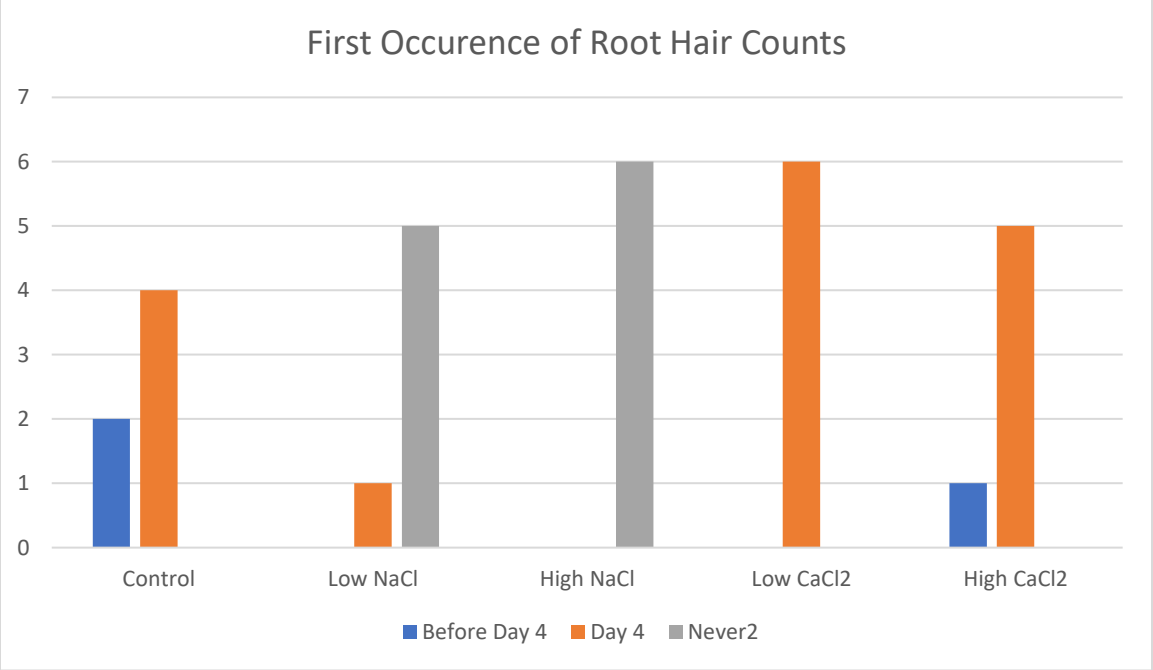

Figure 2. Bar plot of first occurrence of root hair counts.

Results

A Fisher's exact test demonstrated an association between treatment and first root hair occurrence ($p<0.001$). It can be concluded that at least two of the treatments differ in their effect on root hair occurrence. Post-hoc multiple comparisons are summarized in Table 2.

Table 2. Multiple comparisons of all ten treatment combinations.

Treatments Compared	P-Value
1 vs 2	0.012
1 vs 3	<0.001*
1 vs 4	0.121
1 vs 5	0.505
2 vs 3	0.296
2 vs 4	0.003*
2 vs 5	0.013
3 vs 4	0.001*
3 vs 5	0.002*
4 vs 5	0.296

Notes: Treatments numbered as labelled in Table 1, * indicates significance at 0.5% significance level after Bonferroni adjustment.

Table 2 illustrates that the following four pairs of treatments are significantly different in their effect on the first root hair occurrence, Control and High NaCl, Low NaCl and Low $CaCl_2$, High NaCl and Low $CaCl_2$, and High NaCl and High $CaCl_2$. It is also worth noting that the two pairs Control and Low NaCl and Low NaCl and High $CaCl_2$ while not significantly different, each have small (<0.014) p-values indicating that in an experiment with a larger sample size these comparisons may in fact become significant.

Root Length and Diameter

Descriptive Statistics

Results in Table 3 demonstrate the root length means for the control group and both $CaCl_2$ treatments are noticeably higher than those of both NaCl treatments. The $CaCl_2$ treatments have the largest standard deviation in relation to root length. The root diameter means are similar at both measurement lengths with both NaCl treatments measuring the widest diameters. Standard deviations of root diameter are similar aside from treatments 4 for the 0.5mm measurement and treatments 3 and 4 for the 1mm measurement. Figure 3. through 5. illustrate the differences in root length, root diameter at 0.5mm of growth and root diameter at 1mm of growth between the treatments.

Table 3. Root length and diameter at day four descriptive statistics.

Treatment	**Root Length**		**Root Diameter at 0.5mm**		**Root Diameter at 1mm**	
	Mean	SD	Mean	SD	Mean	SD
1 (Control)	12.636	1.878	0.221	0.079	0.220	0.030
2 (Low NaCl)	4.958	1.720	0.394	0.083	0.434	0.075
3 (High NaCl)	2.892	0.727	0.462	0.110	0.569	0.143
4 (Low $CaCl_2$)	13.414	4.353	0.275	0.195	0.339	0.262
5 (High $CaCl_2$)	12.460	2.690	0.218	0.054	0.236	0.051

Notes: All measurement units are millimeters. All descriptive statistics in table are from the last day of measurements.

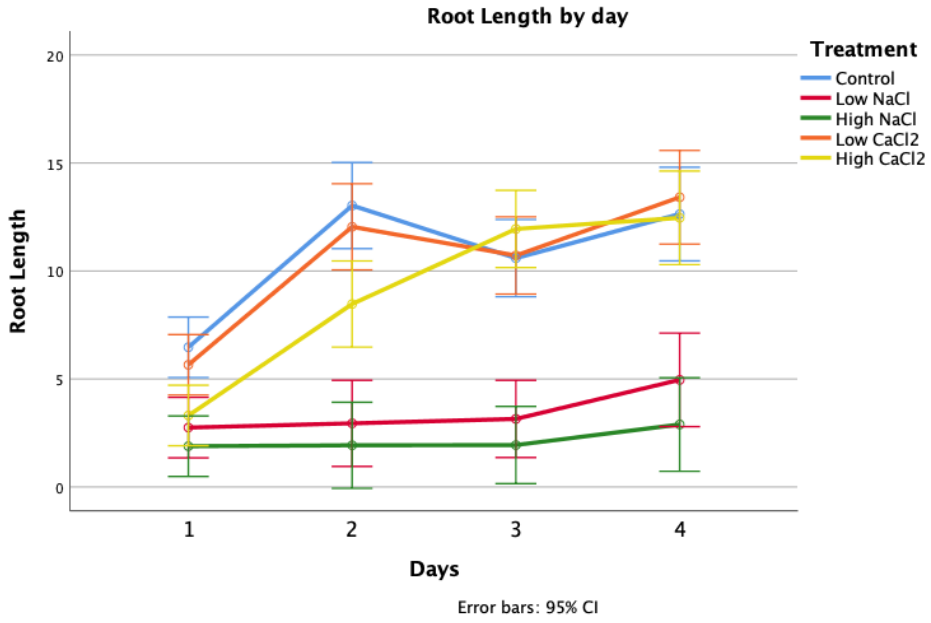

Figure 3. Line graph of root length over day by treatment.

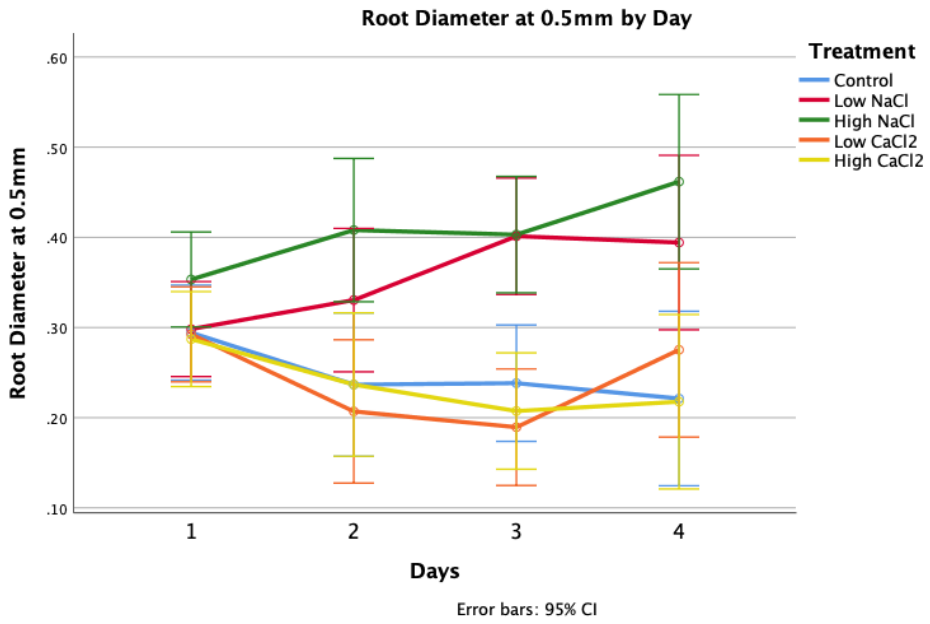

Figure 4. Line graph of diameter at 0.5mm over day by treatment.

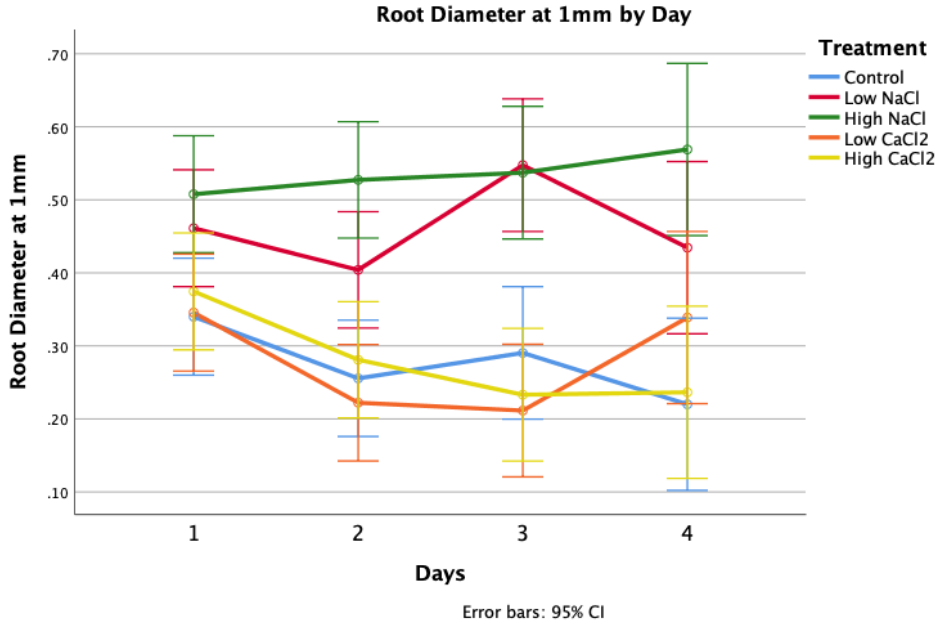

Figure 5. Line graph of diameter at 1mm over day by treatment.

Results

The results of the repeated measure ANOVA summarized in Table 4 show that treatment ($F(4, 25) = 55.918$, $p < 0.001$), day ($F(3, 23) = 58.865$, $p < 0.001$), and the interaction between treatment and day ($F(12, 61.144) = 5.661$, $p < 0.001$), are all significant in their effect on root

length. Partial eta squared is a measure of effect size used to evaluate the proportion of variation explained by a given predictor. Treatment had the largest effect size (0.899), followed by day (0.885) and then their interaction (0.483). The post-hoc multiple comparison summarized in Table 5 shows that the treatments Control, Low $CaCl_2$, and High $CaCl_2$ do not differ in their effect on root length. Furthermore, Low NaCl and High NaCl do not differ in their effect on root length. However, the treatments Control, Low $CaCl_2$, and High $CaCl_2$ all differ in their effect on root length when compared to the treatments Low NaCl and High NaCl. Lastly a post-hoc multiple comparison revealed that days 2, 3 and 4 did not differ in their effect on root length but the effect of day 1 differs from that of the other three days.

Table 4. Root length repeated measure ANOVA results.

Effect	F	df1, df2	P-Value	Partial Eta Squared
Treatment	55.918	4, 25	<0.001	0.899
Day	58.865	3, 23	<0.001	0.885
Treatment and Day Interaction	5.661	12, 61.144	<0.001	0.483

Table 5. Root length post-hoc multiple comparisons for treatments.

Treatments Compared	**P-Value**
1 vs 2	<0.001*
1 vs 3	<0.001*
1 vs 4	1
1 vs 5	0.427
2 vs 3	1
2 vs 4	<0.001*
2 vs 5	<0.001*
3 vs 4	<0.001*
3 vs 5	<0.001*
4 vs 5	0.771

Notes: Treatments numbered as labelled in Table 1, * indicates significance at 0.5% significance level after Bonferroni adjustment.

Root Diameter at 0.5mm of Growth

From Table 6 it is evident that the effect of treatment ($F(4, 25) = 17.531$, $p < 0.001$) and the interaction between treatment and day ($F(12, 61.144) = 1.922$, $p = 0.049$) are significant in their effect on root diameter at 0.5mm of growth while day by itself ($F(3, 23) = 0.947$, $p = 0.434$) is not. Treatment had the largest effect size (0.737), followed by the interaction between day and treatment (0.246) and then day (0.110). Similar to the results from root length, Table 7 shows the Control and both $CaCl_2$ treatments are not significantly different in their effect on root diameter at 0.5mm of growth and both NaCl treatments do not differ in their effect. Once again when compared, both of these groups are significantly different in their effects on root diameter.

Table 6. Root diameter at 0.5mm of growth repeated measure ANOVA results.

Effect	F	df1, df2	P-Value	Partial Eta Squared
Treatment	17.531	4, 25	<0.001	0.737
Day	0.947	3, 23	0.434	0.110
Treatment and Day Interaction	1.922	12, 61.144	0.049	0.246

Table 7. Root diameter at 0.5mm of growth post-hoc multiple comparisons for treatments.

Treatments Compared	**P-Value**
1 vs 2	0.004*
1 vs 3	<0.001*
1 vs 4	1
1 vs 5	1
2 vs 3	0.684
2 vs 4	0.002*
2 vs 5	0.001*
3 vs 4	<0.001*
3 vs 5	<0.001*
4 vs 5	1

Notes: Treatments numbered as labelled in Table 1, * indicates significance at 0.5% significance level after Bonferroni adjustment.

Root Diameter at 1mm of Growth

From Table 8, for root diameter at 1mm, both treatment ($F(4, 25) = 18.927$, $p < 0.001$) and day ($F(3, 23) = 3.516$, $p = 0.031$) are significant in their effects while their interaction ($F(12, 61.144) = 1.749$, $p = 0.078$) is not. Treatment had the largest effect size (0.752), followed by day (0.314) and then their interaction (0.229). The results of the multiple comparison in Table 9. are consistent with both previous results. A post-hoc comparison of the effect of the days revealed that at the significance level of 0.83%, as calculated from the Bonferroni correction, none of the days significantly differ in their effect on root diameter at 1mm of growth.

Table 8. Root diameter at 1mm of growth repeated measure ANOVA results.

Effect	F	df1, df2	P-Value	Partial Eta Squared
Treatment	18.927	4, 25	<0.001	0.752
Day	3.516	3, 23	0.031	0.314
Treatment and Day Interaction	1.749	12, 61.144	0.078	0.229

Table 9. Root diameter at 1mm of growth post-hoc multiple comparisons for treatments.

Treatments Compared	**P-Value**
1 vs 2	0.001*
1 vs 3	<0.001*
1 vs 4	1
1 vs 5	1
2 vs 3	0.780
2 vs 4	0.001*
2 vs 5	0.001*
3 vs 4	<0.001*
3 vs 5	<0.001*
4 vs 5	1

Notes: Treatments numbered as labelled in Table 1, * indicates significance at 0.5% significance level after Bonferroni adjustment.

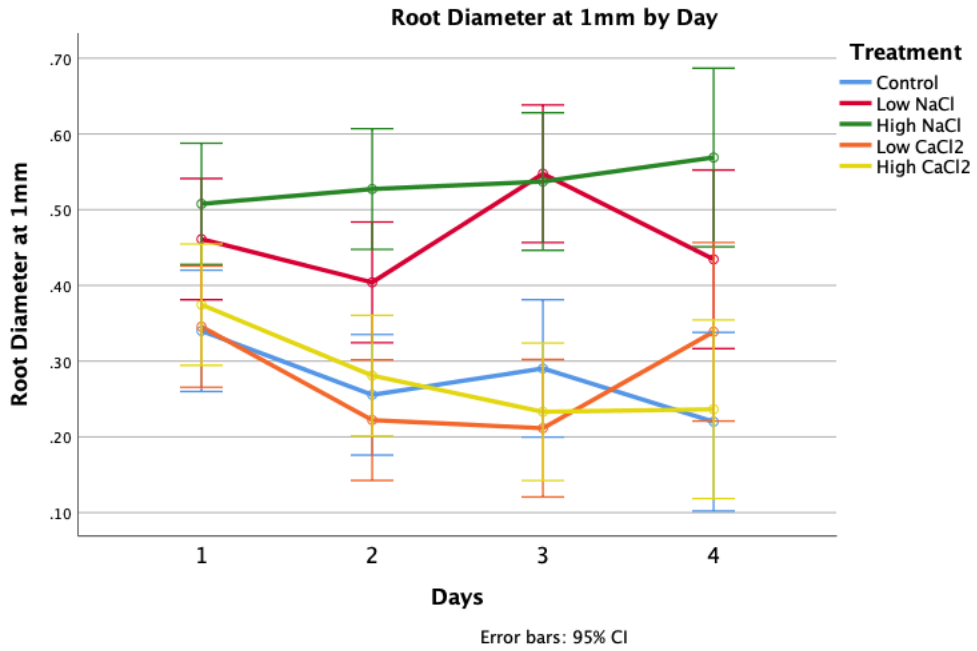

Figure 5. Line graph of diameter at 1mm over day by treatment.

Germination

Descriptive Statistics

Table 10. is composed of 1800 observations, one for all thirty pots in each of the six treatments on all ten days. For every treatment the cells represent the number of times a specific germination count was recorded totalling 300 as the thirty pots were observed on ten different days. The most common number of plants germinated among the control pots was four. The Low $CaCl_2$ treatment saw two as the most common number of plants germinated among pots. For all other treatments zero plants germinated was the most common observation. Furthermore treatment 3, high NaCl, only had 32 observations that had plants germinating.

Table 10. Germination count totals from all ten measurement days.

Treatment	**Germination Counts**					
	0	**1**	**2**	**3**	**4**	**5**
1 (Control)	4	7	35	66	126	62
2 (Low NaCl)	108	66	78	24	19	5
3 (High NaCl)	268	28	4	0	0	0
4 (Low $CaCl_2$)	54	69	83	43	39	12
5 (High $CaCl_2$)	169	73	34	13	11	0
6 (Combination of Both Salts)	198	60	26	1	15	0

Results

A logistic regression modelling the probability to germinate in dependency on day, treatment and their interaction revealed treatment was a significant predictor ($\chi^2(5) = 2049.943$, $p < 0.001$) in its effect on seed germination. Measurement day ($\chi^2(9) = 1084.705$, $p < 0.001$) and the interaction between treatment and day ($\chi^2(43) = 902.226$, $p < 0.001$) were also both significant in their effects. The deviance/df (< 2 indicates good model fit) of the fitted model (1.421) as well as the Omnibus test ($p < 0.001$) confirm that the logistic regression model fit is appropriate.

Table 11. Logistic regression model effects for germination data.

Effect	Wald Chi-Squared	df	P-Value
Treatment	2049.943	5	<0.001
Day	1084.705	9	<0.001
Treatment and Day Interaction	902.226	43	<0.001

A post-hoc multiple comparison, available in the appendix (A1), determined that all pairs of treatments differ from each other in their effect on seed germination rate except the pair of treatments 5 and 6. A separate post-hoc multiple comparison was conducted for all pairs of days. Many of the days differed their effects on seedling germination. Results of the multiple comparisons are summarized in Figure 6. It is clear from the figure that the high NaCl is the most detrimental to germination followed by the combination of both salts, the high $CaCl_2$

treatment and then low NaCl. Consistent with the hypothesis, the low $CaCl_2$ treatment is the least harmful to germination. A full results table of the logistic regression can be found in the appendix (A5).

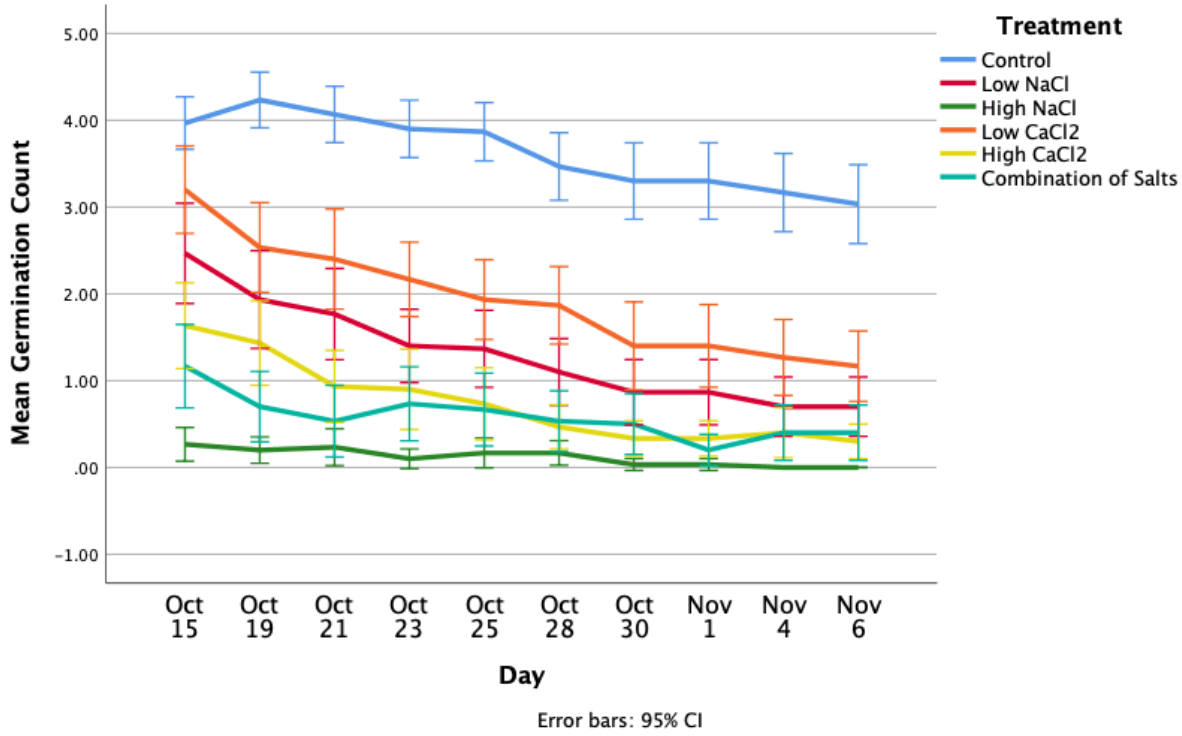

Figure 6. Line graph demonstrating germination counts over measurement days by treatment.

True Leaf Development

Descriptive Statistics

As seen in Table 12. treatments 3, 4 and 6 all showed no leaf development while treatments 2 and 5 had two and three non-zero counts respectively. Due to the fact that only the control showed consistent leaf development it is not appropriate to fit a regression model to the data as was done with germination. However, by viewing the descriptive statistics it is clear that the salt treatments had a detrimental effect on leaf development.

Table 12. True leaf development count totals from both measurement days.

Treatment	**True Leaf Development Counts**					
	0	**1**	**2**	**3**	**4**	**5**
1 (Control)	6	19	14	7	12	2
2 (Low NaCl)	58	1	1	0	0	0
3 (High NaCl)	60	0	0	0	0	0
4 (Low $CaCl_2$)	60	0	0	0	0	0
5 (High $CaCl_2$)	57	3	0	0	0	0
6 (Combination of Both Salts)	60	0	0	0	0	0

Discussion

The researcher's hypothesis that NaCl is a more harmful salt in terms of plant growth and development when compared to $CaCl_2$ was partially supported by the analysis. Root hair occurrence was less frequent in the NaCl treatments. Similarly, root length was longer in the $CaCl_2$ treatments. The low treatment of $CaCl_2$ was responsible for more frequent germination than the low treatment of NaCl. The results were identical for the high treatments. In terms of leaf development, the only treatment with consistent leaf development was the control. These results are likely due to the fact that sodium is toxic at high concentrations while calcium is not. Sodium and chloride ions cause damage to the plant's photosystems reducing the efficiency of photosynthesis. While the NaCl treatments were seen to have higher diameters than the $CaCl_2$ treatments of the same concentrations. The reason for this is likely due to the stunted growth attributed to the NaCl treatments. As a result, the root cap fails to elongate causing the root to be stuck in the initial phases of growth. Therefore, the root stays short and stubby rather than growing longer like in the other treatments. As seen from this analysis it would be beneficial for plants if $CaCl_2$ was used as a de-icing agent rather than NaCl.

Reference:

Kang, J., Zhao, W., Zheng, Y., Zhang, D. M., Zhou, H., & Sun, P. (2017). Calcium chloride improves photosynthesis and water status in the C4 succulent xerophyte Haloxylon ammodendron under water deficit. *Plant Growth Regulation*, *82*(3), 467–478. doi: 10.1007/s10725-017-0273-4

Appendix

Table A1. Germination post-hoc multiple comparisons for treatments.

Treatments Compared	P-Value
1 vs 2	<0.001*
1 vs 3	<0.001*
1 vs 4	<0.001*
1 vs 5	<0.001*
1 vs 6	<0.001*
2 vs 3	<0.001*
2 vs 4	<0.001*
2 vs 5	<0.001*
2 vs 6	<0.001*
3 vs 4	<0.001*
3 vs 5	<0.001*
3 vs 6	<0.001*
4 vs 5	<0.001*
4 vs 6	<0.001*
5 vs 6	1

Notes: Treatments numbered as labelled in Table 10, * indicates significance at 0.33% significance level after Bonferroni adjustment.

Table A2. Logistic regression results with germination as response.

Parameter	Coefficient	SE	Wald-Chi Squared	df	P-Value
Intercept	-2.442	0.301	65.854	1	<0.001
(1) Control	2.879	0.344	69.774	1	<0.001
(2) Low NaCl	0.627	0.382	2.694	1	0.101
(3) High NaCl	-31.091	1.086	818.983	1	<0.001
(4) Low $CaCl_2$	1.253	0.3578	12.276	1	<0.001
(5) High $CaCl_2$	-0.209	0.457	0.458	1	0.499
(a) Oct 15	1.253	0.358	12.276	1	<0.001

(b) Oct 19	0.627	0.382	2.694	1	0.101
(c) Oct 21	0.317	0.401	0.626	1	0.429
(d) Oct 23	0.681	0.379	3.227	1	0.072
(e) Oct 25	0.571	0.385	2.195	1	0.138
(f) Oct 28	0.317	0.401	0.626	1	0.429
(g) Oct 30	0.245	0.406	0.365	1	0.546
(h) Nov 1	-0.736	0.514	2.049	1	0.152
(i) Nov 4	<0.001	0.426	0	1	1
(1)*(a)	-0.341	0.443	0.592	1	0.442
(1)*(b)	0.648	0.475	1.866	1	0.172
(1)*(c)	0.721	0.482	2.239	1	0.135
(1)*(d)	0.151	0.459	0.108	1	0.742
(1)*(e)	0.223	0.463	0.233	1	0.629
(1)*(f)	0.065	0.469	0.019	1	0.889
(1)*(g)	-0.015	0.472	0.001	1	0.974
(1)*(h)	0.966	0.567	2.898	1	0.089
(1)*(i)	0.113	0.488	0.054	1	0.816
(2)*(a)	0.536	0.458	1.368	1	0.242
(2)*(b)	0.727	0.479	2.303	1	0.129
(2)*(c)	0.894	0.495	3.259	1	0.071
(2)*(d)	0.189	0.482	0.155	1	0.694
(2)*(e)	0.267	0.487	0.300	1	0.584
(2)*(f)	0.233	0.505	0.212	1	0.645
(2)*(g)	0.008	0.516	0	1	0.988
(2)*(h)	0.989	0.605	2.671	1	0.102
(2)*(i)	<0.001	0.540	0	1	1
(3)*(a)	29.404	1.162	640.634	1	<0.001
(3)*(b)	29.728	1.187	627.104	1	<0.001
(3)*(c)	30.200	1.183	651.374	1	<0.001
(3)*(d)	28.960	1.254	532.942	1	<0.001
(3)*(e)	29.596	1.202	606.201	1	<0.001

(3)*(f)	29.849	1.207	611.436	1	<0.001
(3)*(g)	28.284	1.504	353.814	1	<0.001
(3)*(h)	29.265	-	-	-	-
(3)*(i)	-0.003	-	-	-	-
(4)*(a)	0.512	0.441	1.352	1	0.245
(4)*(b)	0.589	0.458	1.654	1	0.198
(4)*(c)	0.792	0.474	2.797	1	0.094
(4)*(d)	0.240	0.456	0.276	1	0.599
(4)*(e)	0.158	0.462	0.116	1	0.733
(4)*(f)	0.355	0.476	0.555	1	0.456
(4)*(g)	<0.001	0.485	0	1	1
(4)*(h)	0.981	0.578	2.876	1	0.090
(4)*(i)	0.109	0.504	0.047	1	0.829
(5)*(a)	0.775	0.526	2.176	1	0.140
(5)*(b)	1.213	0.545	4.957	1	0.026
(5)*(c)	0.963	0.568	2.872	1	0.090
(5)*(d)	0.554	0.554	0.988	1	0.318
(5)*(e)	0.420	0.566	0.552	1	0.458
(5)*(f)	0.161	0.598	0.072	1	0.788
(5)*(g)	-0.133	0.625	0.045	1	0.832
(5)*(h)	0.848	0.700	1.470	1	0.225
(5)*(i)	0.309	0.625	0.245	1	0.621

Notes: Reference categories are combination of salts and Nov 6. All values are rounded to three decimals. - represent cells with no output from SPSS