Brian Tomasik

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Comparison of the Toxicity of Sodium Chloride

and Calcium Chloride on Buttercrunch Lettuce Seeds

Introduction

Sodium chloride, NaCl, also known as rock salt, is the most common and least expensive deicing chemical applied to roads during the winter. A less common deicing agent is calcium chloride, $CaCl_2$; this chemical is about three times more expensive and harder to handle than sodium chloride, but it is also more effective and continues working at lower temperatures.

The present investigation will test the following hypotheses regarding these two salts by means of a lettuce-seed bioassay: that both of them adversely impact roadside vegetation and that calcium chloride is more benign to such vegetation than sodium chloride. Lettuce seeds are commonly used to measure the overall toxicology of environmental contaminants and should indicate with fair accuracy the relative impacts of sodium chloride and calcium chloride on roadside plants.

Methodology and Procedure

(1) Preparing the seeds. I purchased four bags of seeds of buttercrunch lettuce (*Lactuca sativa L.*). Because the origins of the seeds or the conditions of storage in each of the bags may have been different, I mixed all four bags together so that these factors would not act as confounding variables. Just before putting the seeds in Petri dishes, I soaked them for twenty

minutes in a ten-percent bleach solution (one part household bleach and nine parts deionized water) in order to kill fungal spores. Then I filtered the solution away and rinsed the seeds five times with deionized water.

(2) Preparing the solutions. I wanted to be able to determine whether each of the salts inhibited germination and radicle growth more than plain water, as well as whether one salt was more toxic than the other. Therefore, I created nine solutions: pure deionized water, 0.001-M NaCl, 0.001-M $CaCl_2$, 0.01-M NaCl, 0.01-M $CaCl_2$, 0.1-M NaCl, 0.1-M $CaCl_2$, 1-M NaCl, and 1-M $CaCl_2$. Comparing each of the salt solutions against the control would test whether the salt was more toxic than pure water, and comparing the two salt solutions of equal concentration with one another would indicate whether one salt was more toxic than the other at that concentration.

(3) Preparing the Petri dishes. Using tongs, I put one round piece of filter paper into each of 27 dishes. Then I added 2 mL of each solution to three different dishes and placed ten seeds into each dish so that they would have maximum space between them. I put the dishes into large plastic bags, sealed off the ends of the bags with twist-ties, and fit the bags into a long, thin box that I taped shut to keep the seeds in the dark. After eight days, I opened the box and recorded the results.

(4) Taking measurements. For each of the nine solutions, I measured the proportion of seeds that had germinated and the radicle lengths of the germinating seeds. I considered a seed to have germinated if a shoot had broken through the seed coat, even if no distinct radicle was visible. I measured the length of the radicle (Figure 1) by stretching it out along the edge of a ruler.

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Figure 1, from "Collecting and Interpreting Lettuce Seed Bioassay Data," Environmental Inquiry, Cornell University, 30 May 2005 <http://ei.cornell.edu/toxicology/bioassays/lettuce/data.asp>.

Results

Table 1
Proportion of Seeds Germinated and Radicle Lengths for Control Solution

	Proportion Germinated	Individual Radicle Lengths of Germinated Seeds (cm)
Control (deionized water)	28/30	7.70, 6.30, 6.20, 6.10, 6.05, 6.00, 5.85, 5.30, 5.00, 4.70, 4.30, 3.40, 3.30, 3.10, 3.05, 3.00, 2.70, 2.55, 2.10, 1.95, 1.70, 1.20, 0.70, 0, 0, 0, 0, 0

Table 2
Proportion of Seeds Germinated and Radicle Lengths for 0.001-M Solution

	Proportion Germinated	Individual Radicle Lengths of Germinated Seeds (cm)
NaCl (0.001 M)	28/30	7.00, 6.70, 6.60, 5.90, 5.80, 5.25, 5.25, 5.10, 4.75, 4.30, 4.00, 3.35, 3.05, 2.95, 2.70, 2.15, 1.80, 1.65, 1.20, 1.15, 0.90, 0.90, 0.80, 0.70, 0.30, 0.25, 0.10, 0
$CaCl_2$ (0.001 M)	29/30	7.35, 7.30, 6.70, 6.60, 6.10, 5.80, 5.80, 5.70, 5.60, 5.10, 5.00, 5.00, 4.85, 4.50, 4.40, 4.20, 4.10, 3.70, 3.60, 3.50, 2.75, 2.30, 1.80, 1.50, 0.95, 0.70, 0.70, 0, 0

Table 3
Proportion of Seeds Germinated and Radicle Lengths for 0.01-M Solution

	Proportion Germinated	Individual Radicle Lengths of Germinated Seeds (cm)
NaCl (0.01 M)	27/30	5.80, 5.10, 4.90, 4.90, 4.80, 4.65, 4.50, 4.50, 4.35, 4.35, 4.35, 3.80, 3.80, 3.55, 3.50, 3.10, 3.10, 2.95, 2.30, 1.90, 1.05, 1.00, 0.80, 0.50, 0.40, 0.40, 0.40
$CaCl_2$	28/30	7.20, 5.75, 5.45, 5.40, 5.40, 5.25, 5.15, 5.10, 5.00, 4.50, 4.00, 3.80,

(0.01 M)		3.70, 3.30, 2.45, 2.25, 1.90, 1.50, 1.45, 1.40, 1.40, 1.00, 0.80, 0.60, 0.30, 0.30, 0.30, 0.15

Table 4
Proportion of Seeds Germinated and Radicle Lengths for 0.1-M Solution

	Proportion Germinated	Individual Radicle Lengths of Germinated Seeds (cm)
NaCl (0.1 M)	29/30	3.20, 3.10, 3.10, 2.95, 2.90, 2.65, 2.60, 2.35, 2.30, 2.30, 2.10, 2.00, 2.00, 2.00, 1.95, 1.80, 1.70, 1.60, 1.60, 1.40, 1.25, 1.20, 1.20, 0.80, 0.55, 0.45, 0.45, 0.40, 0.30
$CaCl_2$ (0.1 M)	23/30	1.70, 1.65, 1.60, 1.45, 1.35, 1.30, 1.20, 1.20, 1.20, 1.15, 1.15, 1.10, 1.00, 1.00, 1.00, 0.95, 0.95, 0.65, 0.55, 0.15, 0.15, 0, 0

Table 5
Proportion of Seeds Germinated and Radicle Lengths for 1-M Solution

	Proportion Germinated	Individual Radicle Lengths of Germinated Seeds (cm)
NaCl (1 M)	0/30	-
$CaCl_2$ (1 M)	0/30	-

Statistical Analysis – Comparing Proportion Germinated for Salt Solutions and Control

Unfortunately, the assumption that *n(p-hat)* and *n(1 – p-hat)* ≥ 5 only held true for the comparison between the 1-M solutions and the control; therefore, this is the only comparison about which I have drawn conclusions using the two-proportion z test. (Because $n_1 = n_2$, I have simply written *n* to represent both at once.)

(1) Control and 1-M NaCl. (a) Definitions. Let “1” represent “control”; let “2” represent “1-M NaCl.” $n_1 = n_2 = 30$. $p\text{-}hat_1 = 28/30$; $p\text{-}hat_2 = 0/30$; *p-hat* = 28/60. (b) Hypotheses. H_0: $p_1 = p_2$. H_a: $p_1 > p_2$. I used a one-sided alternative because I suspected *a priori* that the control solution will have better germination than the 1-M NaCl solution. (c) Assumptions. (i) two independent simple random samples √ (ii) 10*n* ≤ population; 10(30) ≤ population; 300 ≤ population √ (iii) *n(p-hat)* = 30(28/60) = 14 ≥ 5 √ (iv) *n(1 – p-hat)* = 30(32/60) = 16 ≥ 5 √. (d) Results. $z = 7.2457$; $p = 2.1670 \cdot 10^{-13}$. (e) Conclusion. A difference in sample proportions at

least this extreme would practically never happen by chance alone. The null hypothesis is rejected in favor of the alternative, that the rate of germination of the 1-M NaCl solution was lower than that of pure deionized water.

(2) Control and 1-M $CaCl_2$. All of the numbers in this test were exactly the same as those above, because $p\text{-}hat_{NaCl} = p\text{-}hat_{CaCl2} = 0/30$. Thence I concluded that the rate of germination in the 1-M $CaCl_2$ solution was lower than that of the pure deionized water.

Statistical Analysis – Comparing Radicle Length for Salt Solutions and Control

(1) Control and 0.001-M NaCl. (a) Definitions. Let “1” represent “control”; let “2” represent “0.001-M NaCl.” $n_1 = 28$; $n_2 = 28$. $x\text{-}bar_1 = 3.2946$ cm; $x\text{-}bar_2 = 3.0214$ cm. (b) Hypotheses. H_0: $\mu_1 = \mu_2$. H_a: $\mu_1 > \mu_2$. I used a one-sided alternative because I suspected *a priori* that the population mean radicle length of the control solution will be greater than that of the salt solution. (c) Assumptions. (i) two independent simple random samples √ (ii) no clear skew and no outliers in either sample distribution √ (d) Results. $t = 0.4414$; $df = 53.9673$; $p = 0.3303$. (e) Conclusion. A difference at least this extreme would result 33 times in a hundred, meaning that I cannot reject the null hypothesis. I therefore cannot reach any conclusion.

(2) Control and 0.001-M $CaCl_2$. (a) Definitions. Let “1” represent “control”; let “2” represent “0.001-M $CaCl_2$.” $n_1 = 28$; $n_2 = 29$. $x\text{-}bar_1 = 3.2946$ cm; $x\text{-}bar_2 = 3.9862$ cm. (b) Hypotheses. H_0: $\mu_1 = \mu_2$. H_a: $\mu_1 > \mu_2$. (c) Conclusion. It is already obvious that I will have P-value greater than 0.5. A two-sided test gives a P-value of 0.2566, so I can’t establish a statistically significant difference in either direction.

(3) Control and 0.01-M NaCl. (a) Definitions. Let “1” represent “control”; let “2” represent “0.01-M NaCl.” $n_1 = 28$; $n_2 = 27$. $x\text{-}bar_1 = 3.2946$ cm; $x\text{-}bar_2 = 3.1389$ cm. (b)

Hypotheses. H_0: $\mu_1 = \mu_2$. H_a: $\mu_1 > \mu_2$. (c) Assumptions. (i) two independent simple random samples √ (ii) no clear skew and no outliers in either sample distribution √ (d) Results. t = 0.2812; df = 49.6305; p = 0.3899. (e) Conclusion. A difference at least this extreme would occur 39 times in a hundred, so I cannot reject the null hypothesis.

(4) Control and 0.01-M $CaCl_2$. (a) Definitions. Let "1" represent "control"; let "2" represent "0.01-M $CaCl_2$." n_1 = 28; n_2 = 28. $x\text{-}bar_1$ = 3.2946 cm; $x\text{-}bar_2$ = 3.0286 cm. (b) Hypotheses. H_0: $\mu_1 = \mu_2$. H_a: $\mu_1 > \mu_2$. (c) Assumptions. (i) two independent simple random samples √ (ii) no clear skew and no outliers in either sample distribution √ (d) Results. t = 0.4457; df = 53.4777; p = 0.3288. (e) Conclusion. A difference at least this extreme would happen 33 times in a hundred, so I cannot reject the null hypothesis.

(5) Control and 0.1-M NaCl. (a) Definitions. Let "1" represent "control"; let "2" represent "0.1-M NaCl." n_1 = 28; n_2 = 29. $x\text{-}bar_1$ = 3.2946 cm; $x\text{-}bar_2$ = 1.8000 cm. (b) Hypotheses. H_0: $\mu_1 = \mu_2$. H_a: $\mu_1 > \mu_2$. (c) Assumptions. (i) two independent simple random samples √ (ii) no clear skew and no outliers in either sample distribution √ (d) Results. t = 3.1628; df = 34.3249; p = 0.0016. (e) Conclusion. Results at least this extreme would only happen two times in a thousand. The null hypothesis is rejected in favor of the alternative: the population average root length of the control solution was greater than that of the 0.1-M-NaCl solution.

(6) Control and 0.1-M $CaCl_2$. (a) Definitions. Let "1" represent "control"; let "2" represent "0.1-M $CaCl_2$." n_1 = 28; n_2 = 23. $x\text{-}bar_1$ = 3.2946 cm; $x\text{-}bar_2$ = 0.9761 cm. (b) Hypotheses. H_0: $\mu_1 = \mu_2$. H_a: $\mu_1 > \mu_2$. (c) Assumptions. (i) two independent simple random samples √ (ii) no clear skew and no outliers in either sample distribution √ (d) Results. t = 5.0913; df = 30.0265; $p = 8.9809 \cdot 10^{-6}$. (e) Conclusion. A difference at least this extreme

would practically never happen by chance alone. The null hypothesis is rejected, and I can conclude that the lettuce-seed radicles were inhibited by the 0.1-M $CaCl_2$ solution.

Statistical Analysis – Comparing NaCl and $CaCl_2$

Again, because the assumption that both *n(p-hat)* and *n(1 – p-hat)* ≥ 5 is not true, I cannot perform any two-proportion z tests.

(1) 0.001-M NaCl and 0.001-M $CaCl_2$. (a) Definitions. Let “1” represent “0.001-M NaCl”; let “2” represent “0.001-M $CaCl_2$.” $n_1 = 28$; $n_2 = 29$. $x\text{-}bar_1 = 3.0214$ cm; $x\text{-}bar_2 =$ 3.9862 cm. (b) Hypotheses. H_0: $\mu_1 = \mu_2$. H_a: $\mu_1 < \mu_2$. I used a one-sided alternative because I suspected *a priori* that NaCl would inhibit radicle growth more than $CaCl_2$. (c) Assumptions. (i) two independent simple random samples √ (ii) no clear skew and no outliers in either sample distribution √ (d) Results. $t = -1.6207$; $df = 54.7103$; $p = 0.0554$. (e) Conclusion. Because a difference at least this extreme would occur more than five times in a hundred, I cannot reject the null hypothesis.

(2) 0.01-M NaCl and 0.01-M $CaCl_2$. (a) Definitions. Let “1” represent “0.01-M NaCl”; let “2” represent “0.01-M $CaCl_2$.” $n_1 = 27$; $n_2 = 28$. $x\text{-}bar_1 = 3.1389$ cm; $x\text{-}bar_2 = 3.0286$ cm. (b) Hypotheses. H_0: $\mu_1 = \mu_2$. H_a: $\mu_1 < \mu_2$. (c) It is already clear that the P-value will be greater than 0.5. Because a two-sided test gives a P-value of 0.8332, I cannot conclude that there is a statistically significant difference in either direction.

(3) 0.1-M NaCl and 0.1-M $CaCl_2$. (a) Definitions. Let “1” represent “0.1-M NaCl”; let “2” represent “0.1-M $CaCl_2$.” $n_1 = 29$; $n_2 = 23$. $x\text{-}bar_1 = 1.8000$ cm; $x\text{-}bar_2 = 0.9761$ cm. Already it is clear that I won’t be able to conclude that $\mu_1 < \mu_2$. Therefore, I’ll use a two-sided alternative instead. (b) Hypotheses. H_0: $\mu_1 = \mu_2$. H_a: $\mu_1 \neq \mu_2$. (c) Assumptions. (i) two

independent simple random samples √ (ii) no clear skew and no outliers in either sample distribution √ (d) Results. $t = 4.2177$; $df = 45.8818$; $p = 1.1486 \cdot 10^{-4}$. (e) Conclusion. A difference at least this extreme would barely ever be the result of chance alone. Hence, there is a statistically significant difference between the population mean radicle length of NaCl and that of $CaCl_2$, and the former is clearly larger than the latter.

Conclusions

Both sodium chloride and calcium chloride did in fact adversely impact lettuce seeds when compared against the control solution. At 1-M concentrations of each salt (the only concentration at which I was able to perform a test), germination was inhibited, while at 0.1-M concentrations of each salt (the only concentration at which I could establish statistical significance), the population mean radicle length of the germinating seeds was diminished.

The relative toxicity of the two salts was less clear. At 0.001 M, the calcium chloride showed strong signs of being less harmful than the sodium chloride (though I could not prove anything definitively, as the P-value was 0.0554). In contrast, at 0.1 M, the calcium chloride was significantly more harmful.

If I were to do this experiment again, I would focus on concentrations around 0.1 M, because it was there and only there that I found statistically significant differences in radicle length (both from the control and between the two salts). I would also increase the sample size at each concentration so that I would be more likely to establish statistical significance and so that *n(1 – p-hat)* would be more likely to equal or exceed five, allowing me to compare the proportions of seeds that germinated.