"How does increasing concentrations of salinity (0,0.1,0.2,0.3,0.4mol L^{-1}) affect the number of germinated seeds of *Salicornia europaea* and *Triticium aestivium* seeds in 7 days as an indication of rate of growth and germination?"

Word Count: 3926

Introduction

One of the greatest limitations of a crop is caused by salinity in water and soil. [1] Salinity in soil is caused by soluble salt mixing with underground water, and as groundwater rises to the surface through capillary, water vaporizes, and thus salt accumulates on soil.[2] Salt accumulation is affecting more than %20 of cultivated land worldwide and at a 10% rate. It is increasing annually for diverse reasons, such as high surface evaporation, low precipitation, saline water irrigation and insufficient cultural practices. It is anticipated to increase by up to 50% by 2050.[3] Salinity harms seed germination, delays plant development, decreases crop yield, and reduces food availability.[4] Therefore, more sustainable sources of food like halophytes, which are naturally evolved salt-tolerant plants, are seen as a choice. They have been reported to be able to tolerate up to 0.5 mol salinity, as in the case of *Salicornia europaea*. [5]

Germination is the term used to describe a seedling's sprouting from a seed and also the process of its metabolic machinery's reactivation. Germination of a seed is dependent on external and internal factors. The external conditions include temperature, oxygen, light or darkness, and water.[6] Salinity's effect on germination is caused either by restricting the supply of water (osmotic effect) or by specific injury to certain metabolic processes (ionic effect).[7]

[1]Shannon, Michael C. "Adaptation of Plants to Salinity." Advances in Agronomy, 1997, pp. 75–120., doi:10.1016/s0065-2113(08)60601-x.
[2] Shrivastava, Pooja, and Rajesh Kumar. "Soil Salinity: A Serious Environmental Issue and Plant Growth Promoting Bacteria as One of the Tools for Its Alleviation." Saudi Journal of Biological Sciences, vol. 22, no. 2, 2015, pp. 123–131., doi:10.1016/j.sjbs.2014.12.001.
[3]Jamil, A., et al. "Gene Expression Profiling of Plants under Salt Stress." Critical Reviews in Plant Sciences, vol. 30, no. 5, 2011, pp. 435–458., doi:10.1080/07352689.2011.605739.
[4] Greenway, H, and R Munns. "Mechanisms of Salt Tolerance in Nonhalophytes." Annual Review of Plant Physiology, vol. 31, no. 1, 1980, pp. 149–190., doi:10.1146/annurev.pp.31.060180.001053.
[5] Flowers, Timothy J., and Timothy D. Colmer. "Salinity Tolerance in Halophytes*." New Phytologist, vol. 179, no. 4, 2008, pp. 945–963., doi:10.1111/j.1469-8137.2008.02531.x.
[6] Raven, Peter H., et al. Biology of Plants. W.H. Freeman, 1987. pp. 504–508.
[7] HARDEGREE, STUART P., and WILLIAM E. EMMERICH. "Partitioning Water Potential and Specific Salt Effects on Seed Germination of Four Grasses." Annals of Botany, vol. 66, no. 5, 1990, pp. 587–595., doi:10.1093/oxfordjournals.aob.a088068.

High levels of salinity damage homeostasis in ion distribution and water potential at whole plant and cellular levels, which results in molecular damage, growth arrest, and even death.[8] However, halophytes' dry weight increases with electrolytes at higher levels than those usually present in normal soil.[9] Halophytes are able to obtain a sufficient amount of water and adapt themselves to high levels of salinity by osmotic adjustment. Halophytes accumulate ions that increase the osmotic potential in their tissue, which permits moisture to move from soil into tissue.

Salicornia is from the Amaranthaceae family, a halophyte genus. *Salicornia europaea*, also known as common glasswort, is commonly found at the edges of wetlands, mudflats, and seashores. *Salicornia europaea* has been reported to be able to tolerate about 0.5 mol/l of salinity.[10] On the other hand, *Triticium aestivium*, from the Poaceae family, Triticum genus, also known as common wheat, is reported to be only tolerant to low salinity.[11] It is also one of the greatest major crops, so with increasing salt accumulation, seeking alternative crop options is important.[12]

I regarded the issue worthy of investigation and conducted it with *Salicornia europaea* and *Triticium aestivium* seeds specifically to compare a high salt tolerant specie, with a specie that is moderate salt-tolerant to see the variance to different salt concentrations for myself and test whether *Salicarnia europaea* is a more sustainable source of food for increasing salt accumulation worldwide.

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[8] "Seed Germination and Seedling Growth of Suaeda Salsa under Salt Stress." Annales Botanici Fennici, vol. 44, no. 3, Finnish Zoological and Botanical Publishing Board, 2007, pp. 161–69, http://www.jstor.org/stable/23727638.
[9] Flowers, T. J., et al. "Halophytes." The Quarterly Review of Biology, vol. 61, no. 3, 1986, pp. 313–337., doi:10.1086/415032.
[10] Yamamoto, Kosuke, et al. "Molecular Cloning Ofacetylcholinesterasegene Fromsalicornia Europaeal." Plant Signaling & Behavior, vol. 4, no. 5, 2009, pp. 361–366., doi:10.4161/psb.4.5.8360.
[11] Mer, R. K., et al. "Effect of Salts on Germination of Seeds and Growth of Young Plants of Hordeum Vulgare, Triticum Aestivum, Cicer Arietinum and Brassica Juncea." Journal of Agronomy and Crop Science, vol. 185, no. 4, 2000, pp. 209–217., doi:10.1046/j.1439-037x.2000.00423.x.
[12]Iizumi, Toshichika, and Toru Sakai. "The Global Dataset of Historical Yields for Major Crops 1981–2016." Scientific Data, vol. 7, no. 1, 2020, doi:10.1038/s41597-020-0433-7.

The aim of the essay is to evaluate whether *Salicornia europaea* is more salt tolerant than *Triticium aestivium*, as the sources from my background research indicate. If so, it will allow me to suggest *Salicornia europaea* as a choice of crop that could be grown more sustainably during salinity increases than major crops like *Triticium aestivium* (common wheat). It is significant as major crops could be greatly damaged by salt accumulation during germination and growth processes.

According to this information, this investigation aims to search for an answer to the research question **"How does increasing concentrations of salinity (0,0.1,0.2,0.3,0.4 mol) affect the number of germinated seeds of *Salicornia europaea* and *Triticium aestivium* seeds in 7 days as an indication of rate of growth and germination?"**

Hypothesis

The main causes of salt damage on germination are osmotic and ionic effects. High levels of salt cause disruption of homeostasis in the ion distribution of cellular plant levels and also in plant water potential. *Salicarnia europaea* is expected to be able to perform adjustments by accumulating Na+, Mg2+, Cl−, NO3-, and SO42-ions, increasing the osmotic potential in tissues and thus allowing the plant to obtain water from the soil.

As they can adapt themselves to higher levels of salinity than most species, I predict that *Salicarnia europaea* will be able to tolerate the increasing salt concentrations better than *Triticium aestivium*, the crop with moderate salt tolerance that cannot accumulate ions. According to my background research and my preliminary experiments, I estimate the specie’s seed will be able to germinate at 0.500 mol, the highest concentration of salt in the experiment. However, it might have a lower initial germination percentage than *Triticium aestivium* as it does not germinate in equal amounts in the same conditions when there is no salt, according to my background research. There will be a decrease in germination percentage in this species due to the reduction of water uptake with the osmotic effect, even if seeds are able to reduce it by ion accumulation.

On the other hand, *Triticium aestivium* is a moderately salt-tolerant species as it has adapted to diverse environmental conditions. However, it cannot accumulate ions like halophytes, which increase the osmotic effect and reduce the water uptake of seed by limiting water supply. Thus, I estimate the effects of increasing salt concentrations will be greater on *Salicarnia europaea* even if it could have a greater initial germination percentage when there is no salt. The seeds might not be able to germinate at the highest value of salt concentration in the experiment, 0.500 mol, as *Salicornia europaea* could not adjust its osmotic potential.

Thus, my hypothesis is that *Triticium aestivium* seeds' germination percentages will decrease more than *Salicarnia europaea's* as salinity is increased due to *Triticium aestivium's* lack of *Salicarnia europaea*'s ability to accumulate ions to adjust osmotic potential. For both species, germination percentages will decrease, but *Salicarnia europaea* might be able to germinate in the highest concentration, while *Triticium aestivium* might not be able to.

Method Development

In this experiment, the aim was to compare the salinity tolerance of *Salicarnia europaea* and *Triticium aestivium* and observe the effect of increasing salt concentration on both species. The tolerance of salinity and the effect of increasing salinity are significant for testing due to increasing salinity worldwide, thus to determine the probable effects and possible solutions or alternatives.

From my prior research, I decided to proceed with my research by investigating seed germination ratios and the trends they follow with the changes in salt concentrations. This method was efficient in allowing me to achieve results and do it at home, meaning that I could observe changes frequently.

In a preliminary experiment, I deduced that equal volumes of water of 10 ml could be used for each petri dish for both species, even though the resources I searched for different water uptakes for the species. However, as the magnitudes of uptake were inconsistent across the resources, I did not want to change the volume and cause a possible inaccuracy in the results. Also, as the investigation will be focused on the difference in the rate of decrease in the number of germinated seeds, the change will matter more than the numbers.

For salt, NaCl was chosen as it was convenient in cost and availability. Prior research, which was verified in my preliminary experiments, was coherent with species. The salt concentration was determined according to the salinity values that could be present in the soil, and the constant increase was kept small as the germination was affected even by the chosen values. The number of seeds was limited to 30 due to the cost of *Salicornia europaea*, but it should be enough for the observations according to my preliminary experiment. It was also convenient for calculations of the rate of decrease in germination percentage.

The duration was determined by my controlled experiments and prior research, which led me to the conclusion that it was 7 days.

To prevent possible inaccuracy caused by temperature and light, all petri dishes with seeds were gathered together in the same room at constant temperature.

Glass petri dishes with a 90mm diameter were used as they allowed light from all angles except from the bottom of the petri dish and were also convenient to purchase. The diameter allowed seed to be placed with enough space in between. The number of filter papers was chosen according to the amount of water they can hold.

Method

Independent Variables:

- Salinity by altering the concentration of NaCl salt in water solution (0, 0.1, 0.2, 0.3, 0.4 mol L^{-1})
- Seed species *Salicornia europaea* and *Triticium aestivium*.

Dependent Variables:

- Number of germinated seeds

Controlled Variables

- Volume of water for each solution (200.0±0.1ml)
- Initial number of seeds used for each concentration of salt and each species (30 seeds)
- Time span of the experiment (7 days)
- Temperature seeds are stored (26.5±0.1°)
- Material and size of petri dishes (glass, 9cm radius)
- Filter paper quantity and size (3 filter papers) (9cm radius)
- Type of salt (NaCl)
- Period of light seeds are exposed in a day (12 hours)
- For each species, seeds are obtained from the same supplier
- Tools for measurement [Silvercrest SC-A25 digital scale (±0.01g), graduated cylinder (±0.1ml), thermometer (±0.1°)]

Apparatus

- 180 *Salicornia europaea* seeds
- 180 *Triticium aestivium* seeds
- 1200.0±0.1ml water
- 250.00±0.01g NaCl
- 12 petri dishes with 9cm radius
- 36 filter papers with 9cm radius,
- Parafilm
- Silvercrest SC-A25 (±0.01g)
- Graduated Cylinder (±0.1ml)
- Thermometer (±0.1°)
- 6 Glass jars
- Spoon

Preparation of NaCl Water Solutions

1. Water is divided into 200±0.1ml 6 glass jars for each concentration measured with graduated cylinder
2. One of the glass jars is left without salt
3. For each concentration, mass of NaCl needed for 200±0.1ml is calculated according to NaCl's molar mass of 58,44g/mol
4. Calculated mass is measured using electronic scale and added to the water in one of the jars and repeated until 6 concentrations of salt (0,0.1,0.2,0.3,0.4 mol L^{-1}) is obtained
5. Solutions are stirred with a spoon at 26.5±0.1° until all salt is dissolved evenly
6. To prevent water loss, the solution is kept at 26.5±0.1° and sealed

Preparation of Seeds

1. Seeds are purchased from online sources
2. 12 petri dishes were prepared with three filter papers for each petri dish
3. For each petri dish, 30 seeds of the same species are placed at equal intervals

Experimentation

1. Each day at 7a.m.(GMT+3) each glass jar is sealed off and the solution inside is stirred to be certain salt is dissolved equally and 20±0.1ml is taken using a graduated cylinder and the jar is sealed off again to prevent water loss
2. 10±0.1ml of the solution is added to one of the petri dishes containing one of the species, and the remaining 10±0.1ml is added to the other petri dish containing the other species
3. Petri dishes are sealed with parafilm to prevent the loss of water

4. Steps 1-3 of experimentation is repeated with all concentrations
5. Water treatment is continued for 7 days in the same room at 26.5±0.1° and 8 hours of daylight each day
6. After 7 days, the number of germinated seeds is recorded to an Excel data table for analysis

Raw Data

Table 1. Calculations of Molarity/Concentration of NaCl Solution in 200 ± 0.1ml Water

Volume of Water (L) (±0.0001)	Mass of NaCl (g) (±0.01)	Mol of NaCl in Solution (to 3 S.F. due to 3 S.F. data from mass) (NaCl:58.44 g mol^{-1})	Concentration / Molarity of Solution (mol L^{-1})
0.2000	0.00	0.00	0.000
0.2000	1.17	0.0200 ± % 0.9	0.100
0.2000	2.34	0.0400 ± % 0.4	0.200
0.2000	3.51	0.0601 ± % 0.3	0.301
0.2000	4.68	0.0801 ± % 0.2	0.401
0.2000	5.84	0.0999 ± % 0.2	0.500

Calculations for concentration uncertainty are in the appendix located at the end of the essay.

Table 2. Number of Germinated Seed of *Salicarnia europaea* and *Triticium aestivium* ,With Initial Number of 30 Seeds at 26.5 ± 0.1°, for Corresponding Concentration/Molarity of NaCl Solution in 10 ± 0.1ml Water per Day. (n is equal to number of germinated seed per each cell)

SEED SPECIES	Concentration / Molarity of Solution (mol L^{-1})	NUMBER OF GERMINATED SEEDS $\pm\sqrt{n}$ (n is equal to the number of germinated seeds per each cell and $\pm\sqrt{n}$ is equal to uncertainty for each concentration's number of germinated seeds)
SALICARNIA EUROPAEA	0.000	24
	0.100	22
	0.200	17
	0.301	11
	0.401	6
	0.500	2
TRITICIUM AESTIVIUM	0.000	27
	0.100	21
	0.200	13
	0.301	4
	0.401	0
	0.500	0

Processed Data

Calculations for Germination Percentage

$$Germination\ Percentage = \frac{number\ of\ germinated\ seeds}{total\ number\ of\ seeds} \text{x100}$$

Sample calculation for 0.000mol L^{-1} of *Salicornia europaea:*

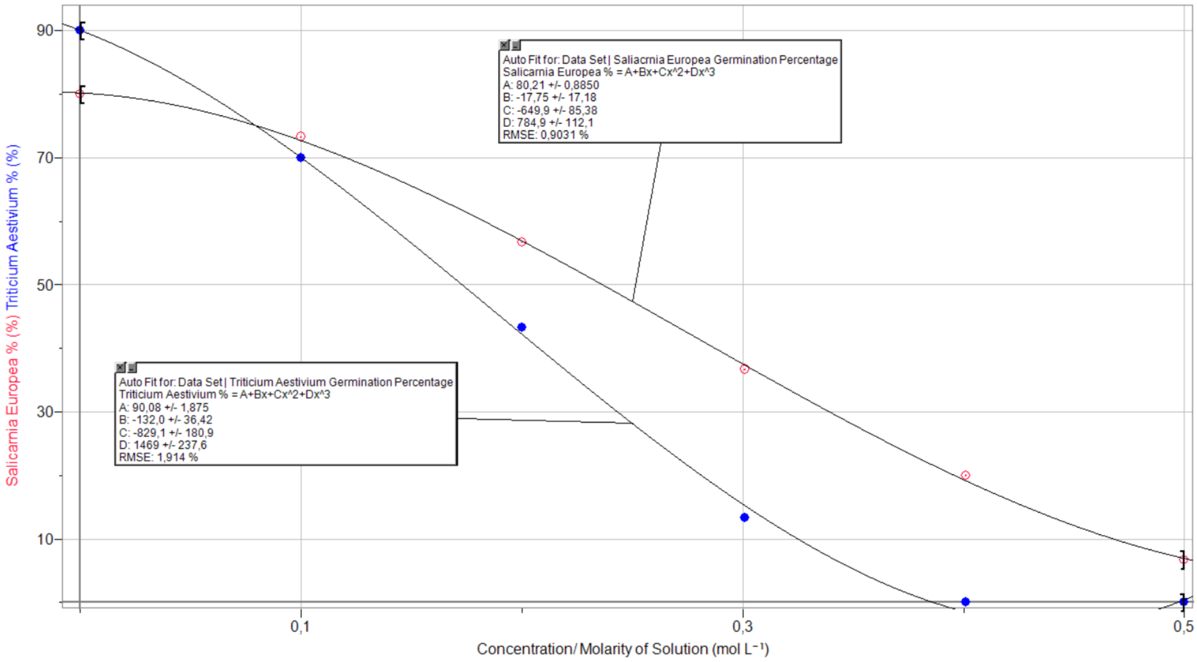
$$Germination\ Percentage = \frac{24}{30}x100$$

=%80

Table 3. Percentage of Germinated Seed of *Salicarnia europaea* and *Triticium aestivium* for Corresponding Concentration/Molarity of NaCl Solution in 10 ± 0.1ml Water per Day

Seed Species	Concentration / Molarity of Solution (mol L^{-1})	*Germination Percentage (%)*
Salicornia europaea	0.000	*80.0*
	0.100	*73.3*
	0.200	*56.7*
	0.301	*36.7*
	0.401	*20.0*
	0.500	*6.7*
Triticium aestivium	0.000	*90.0*
	0.100	*70.0*
	0.200	*43.3*
	0.301	*13.3*
	0.401	*0.0*
	0.500	*0.0*

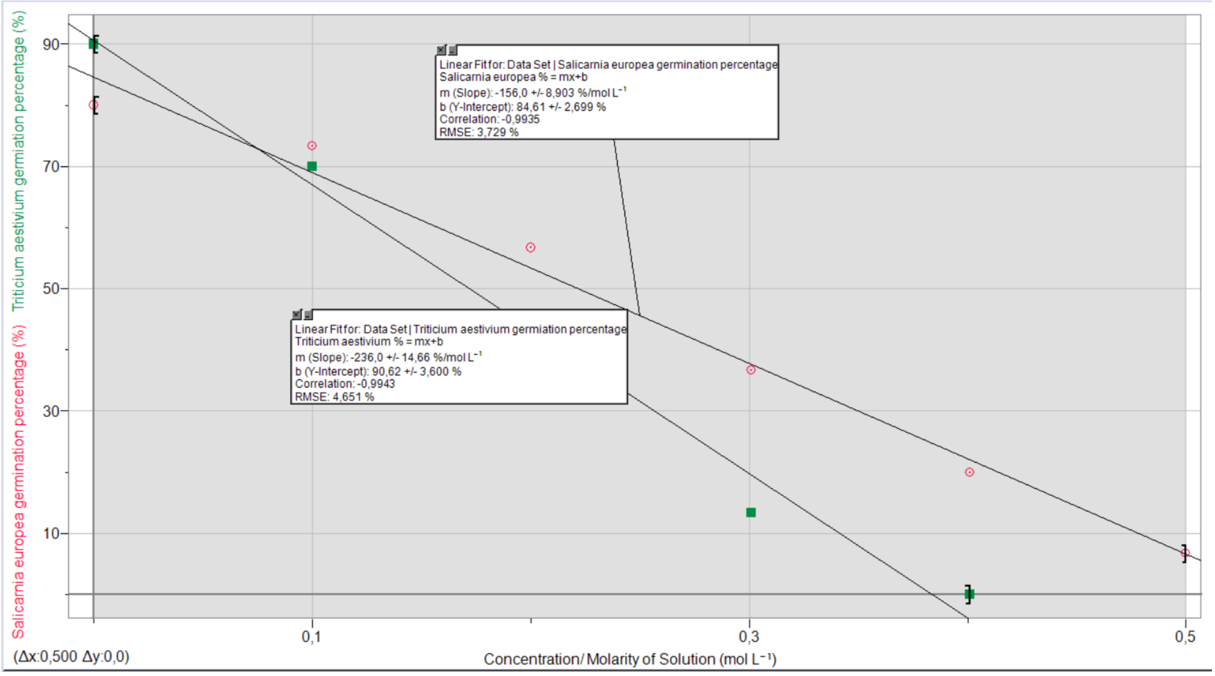
Graph 1. Percentage of Germinated Seed of *Salicarnia europaea* and *Triticium aestivium* Plotted against Corresponding Concentration/Molarity of NaCl Solution in 10 ± 0.1ml Water per Day



The graph shows a decrease in germination percentage for both species, and a greater decrease for *Triticium aestivium*. From the best fit line, *Salicornia europaea* seemed to have a smaller rate of decrease in germination percentage even though it has a smaller initial germination percentage, meaning it was able to tolerate higher salt concentration better than *Triticium aestivium.*

Two methods were used to compare the rates of germination percentage changes. Trying a linear fit in which the slope will give the average rate of change and calculating the average rate of change from the data table.

Graph 2. Linear Fits for Graph 1



According to the linear fit, *Tricium aestivium* has a greater rate of decrease in germination percentage according to the slopes, which means *Triticum aestivium* wasn't able to tolerate the increasing salt concentration like *Salicornia europaea* could.

The average rate of change allows examination of the effect of salt concentrations on the seed with numerical values. A higher rate of change would mean a greater effect on germination as there would be a greater decrease in the number of germinated seeds.

It can be calculated by adding the difference between the germination percentages of consecutive salt concentrations and dividing the sum by the number of differences.

$$Average\ rate\ of\ change =$$

$$\frac{Sum\ of\ differences\ between\ germination\ percentages\ of\ consecutive\ salt\ concentration}{number\ of\ differences}$$

For *Salicornia europaea:*

$$\frac{-(67 + 166 + 200 + 167 + 133)}{5}$$

$$\approx -147$$

For *Triticium aestivium:*

$$\frac{-(200 + 267 + 300 + 133)}{4}$$

$$\approx -225$$

Linear fits gave slopes of -156.0±8.9 and -236.0±14.7 and calculations from the data table of germination percentages gave -147 and -225 for the species germination percentage average change rates. The negative values meant both of the rates were reducing, and the concentration of salt in the solution was inversely proportional with germination percentage for both species. However, the average rate of change of *Triticium aestivium* was greater in magnitude than *Salicornia europaea* as the decrease was much greater and the final germinated seed numbers were zero, which implies that *Triticium aestivium's* salt tolerance is lower than *Salicornia europaea.*

Moreover, a two-way Anova without replication test was applied using the Table 3. data set on Excel to determine the effect of salinity of water and the seed species I chose on the germination percentages by a statistical test. The Two-way Anova test was chosen because it is used to analyze the effects of two independent variables, as in my case, on a dependent variable along with their relationship to the variable.[13] It is used to determine whether the effects on the dependent variable were due to chance or the independent variables.

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[13] Norton, Barbara J., and Michael J Strube. "Guide for the Interpretation of Two-Way Analysis of Variance." Physical Therapy, vol. 66, no. 3, 1986, pp. 402–412., doi:10.1093/ptj/66.3.402.

To determine a statistical relationship and significance in the data, the null hypothesis will be evaluated. A null hypothesis suggests that no significance and no statistical relationship is present between variables. If the P-value found from the test is smaller than α value, which is 0.05, then the null hypothesis is rejected and there is a relationship. If the P-value found from the test is greater than 0.05, then the null hypothesis cannot be rejected, so with %95 level of statistical certainty, it can be concluded that there is no significant difference between the mean germination percentages caused by the independent variables.

There are two null hypotheses in this set of data: one for the specie (rows) and one for the concentrations (columns).

For the rows:

H_0: there is not a statistically significant difference between mean germination percentages caused by using different plant species seeds.

H_1: there is a statistically significant difference between mean germination percentages caused by using different plant species seeds.

For the columns:

H_0: there is not a statistically significant difference between mean germination percentages caused by different concentrations of salt.

H_1: there is a statistically significant difference between mean germination percentages caused by different concentrations of salt.

Figure 1. Two-way Anova Test for Table3. Data Set by Excel

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Salicornia europea	6	2,734	0,455667	0,086433
Triticium aestivium	6	2,166	0,361	0,14465
0.000 Salt Concentration	2	1,7	0,85	0,005
0.100 Salt Concentration	2	1,433	0,7165	0,000545
0.200 Salt Concentration	2	1	0,5	0,008978
0.301 Salt Concentration	2	0,5	0,25	0,027378
0.401 Salt Concentration	2	0,2	0,1	0,02
0.500 Salt Concentration	2	0,067	0,0335	0,002245

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Rows	0,026885	1	0,026885	3,607833	0,115948	6,607891
Columns	1,118156	5	0,223631	30,00981	0,00098	5,050329
Error	0,03726	5	0,007452			
Total	1,182301	11				

Specie	0.000 S	0.100 S	0.200 S	0.301 S	0.401 S	0.500 S
Salicornia	0,80	0,73	0,57	0,37	0,20	0,07
Triticium a	0,90	0,70	0,43	0,13	0,00	0,00

For the rows:

The p-value of the rows: 0.1159 is greater than α-value: 0.05. The null hypothesis cannot be rejected, meaning there is no statistically significant difference between mean germination percentages caused by using different plant species seeds with %95 certainty.

For the columns:

The p-value of the rows: 0.00098 is smaller than α-value: 0.05. The null hypothesis is rejected, meaning there is a statistically significant difference between mean germination percentages caused by different concentrations of salt with %95 certainty.

Conclusion

Graph 1 showed a species difference in the rate of reduction of germinated seed percentages, with *Triticium aestivium* having a steep decrease, indicating a faster rate of reduction. The magnitude was calculated both manually and by creating a linear fit for each species. Both methods gave similar rates in magnitude, while linear fits gave slightly higher values. *Triticium aestivium's* rate was greater in magnitude in both calculations.

Rates of germination change for both species had negative values. While *Triticium aestivium's* was -236.0±14.7 and -225, *Salicornia europaea's* was -156.0±8.9 and -147. *Triticium aestivium* had an initial germination percentage of %90.0 which was reduced to %0.0 by 0.401mol and 0.500mol . The decrease in percentage ranged from %13.3 to %30.0 which was observed between 0.200- 0.301mol. *Triticium aestivium* was able to tolerate salinity until 0.401mol which verifies the knowledge from my research of *Triticium aestivium* being moderate salt tolerant specie.[14] On the other hand, *Salicarnia europaea* showed germination at 0.500mol and the germination percentage decrease ranged from %6.7 to %20.0 between 0.200-0.301mol. Thus, it can be classified as a highly salt tolerant species. 15 Initial germination percentage was %80.0 and inhibited until %6.7.

From the two-way Anova test, the statistical relationship between salt concentration and the germination percentage was approved with % 95 statistical certainty as both species' germination numbers did decrease with greater salt concentration values. However, the statistical significance difference between mean germination percentages caused by using different plant species seeds was rejected with %95 statistical certainty due to the higher values of germination percentage that *Triticium aestivium* had in the initial concentration with no salt and at 0.100mol, which was close to *Salicornia europaea's*. Also, as both of the percentage values were decreasing, the statistically significant difference between mean germination percentages might have been rejected. As *Triticium aestivium* had a greater decrease with no germination at 0.401 and 0.500mol , *Salicornia europaea* was more salt tolerant.

For both species, inhibition of seed germination was observed. As I have stated in my hypothesis, the rate of inhibition and overall reduction of *Triticium aestivium* was greater than

[14] Miyamoto, S., et al. Photo Guide: Landscape Plant Response to Salinity. Agricultural Research and Extension Center at El Paso, Texas Agricultural Experiment Station, the Texas A & M University System, 2004.

Salicornia europaea, which is due to *Salicarnia europaea's* ability to adjust osmotic potential by accumulating ions, according to my research.

Initial numbers of germinated seeds were different, which might be due to temperature, light, and volume of water unintentionally creating more favorable conditions for one species. As I have stated in my method development, water requirements for species seem to be different according to various resources. As there was no common agreement on the amount, it was kept the same for both species. This might be the most significant cause of the different initial germinated seed numbers. Furthermore, as the rates of germination percentage change were examined, the numbers were not very significant for the comparison of the two.

The adjustment of osmotic potential by ion accumulation of halophytes has been tested and confirmed in research by DanQ. Tran, Ayako Konishi, JohnC. Cushman, Masahiro Morokuma, Masanori Toyota &Sakae Agarie(2019)[15] Coherent with my results, Calone, Roberta & Sanoubar, Rabab & Noli, Enrico & Barbanti, Lorenzo. (2020) have also observed a decrease in germination percentage of *Salicornia europaea* seed with increasing salt concentrations[16] Research of H.Akbarimoghaddam, M.alavi, A.Ghanbari, N.Panjehkeh(2011) has also observed a decrease in the germination percentage of *Triticium aestivium* seed with increasing salt concentrations.[17] The concentrations of salt are different between the two articles so no direct comparison can be made between the two species. However; Calone, Roberta & Sanoubar, Rabab & Noli, Enrico & Barbanti, Lorenzo. (2020) have observed seed germination of *Salicornia europaea* up to 0.6mol salt concentration. No direct comparison can be made with the research of H.Akbarimoghaddam, M.Galavi, A.Ghanbari, N.Panjehkeh

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[15] Tran, Dan Q., et al. "Ion Accumulation and Expression of Ion Homeostasis-Related Genes Associated with Halophilism, NaCl-Promoted Growth in a Halophyte Mesembryanthemum Crystallinum L." Plant Production Science, vol. 23, no. 1, 2019, pp. 91–102., doi:10.1080/1343943x.2019.1647788.
[16] Calone, Roberta, et al. "Assessing Salicornia Europaea Tolerance to Salinity at Seed Germination Stage." Agriculture, vol. 10, no. 2, 2020, p. 29., doi:10.3390/agriculture10020029.
[17] ÖNER, Fatih, and Ayşegül KIRLI. "Effects of Salt Stress on Germination and Seedling Growth of Different Bread Wheat (Triticum Aestivum L.) Cultivars." Akademik Ziraat Dergisi, 2018, pp. 191–196., doi:10.29278/azd.476365.

(2011) as different methods were used for salt concentration measurements, which cannot be converted to the unit I have used in my essay (mol).

Evaluations

Evaluation of the Method

The method was successful in giving results that were effective for the investigation. Controlled factors of sunlight and water amount could have caused different initial germination percentage values. However, the change in germination could be evaluated.

Both species showed germination until certain concentrations with NaCl as the salt choice, so it was a convenient choice for sufficient data. However, my results are only based on the effects of NaCl, and the effects of other salt types are unknown, which limits the results of the essay to one salt type. One suggestion to solve this limitation is to investigate the effects of different salts on germination as soil can have various types of salt, including sodium sulfate, magnesium sulfate, calcium chloride, gypsum, and potassium chloride[18]. Another limitation was that the salt concentrations were too high for *Triticium aestivium* as its germination was inhibited after 0.401mol which limited the amount of data for better analysis. To improve the results, the salt concentrations can be reduced as, smaller values of concentration could give more data to be evaluated and smaller values of decrease could increase accuracy due to more detailed values of rate. I only investigated 6 concentrations of salt, which limited the results and accuracy of the rate of change in germination percentage. The number of concentrations could be increased to 10 with smaller differences between the concentrations of about 0.05mol instead of 0.1mol, which would allow better analysis of the effects of the independent variables and could solve the limitation.

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[18] Shrivastava, Pooja, and Rajesh Kumar. "Soil Salinity: A Serious Environmental Issue and Plant Growth Promoting Bacteria as One of the Tools for Its Alleviation." Saudi Journal of Biological Sciences, vol. 22, no. 2, 2015, pp. 123–131., doi:10.1016/j.sjbs.2014.12.001.

A number of seeds were also effective in giving results that could be examined in the investigation. However, larger numbers of seeds are usually used in germination percentage investigations to get more accurate germination percentages as the effects can be examined on a larger scale. I was limited to 30 due to the expense of the seeds. A suggestion is to create more budget and increase the number of seeds to about 50 as in the research of Calone, Roberta & Sanoubar, Rabab & Noli, Enrico & Barbanti, Lorenzo(2020) or different seed species from the halophytes with similar traits for adjusting osmotic potential and less pricey, such as Suaeda, Aeluropus, Thellungiella, Atriplex, Mesembryanthemum, Cakile which have been reported to be salt tolerant[19]

Duration was enough and convenient for the experiment as it gave sufficient data and allowed both species' seeds to germinate. Petri dishes, filter papers and their numbers were also efficient.

Evaluation of Statistical Test Type

The two-way Anova test without repetition was compatible with the set of data obtained as it required two independent variables and one dependent variable. It also allowed me to evaluate the statistical significance of the independent variables' effects on the dependent variable while also allowing me to comment on the conclusion of the results.

Evaluation of Repeatability

With sufficient measures to keep the controlled variables the same and decrease possible outside factors that might cause differences to a minimum, the repeatability of the experiment is high and could give similar values to my research. However, the supplier of seeds may limit the consistency due to different methods of storage and thus the conditions of the seeds. I purchased the seeds from a local supplier. The

[19] Mishra, Avinash, and Bhakti Tanna. "Halophytes: Potential Resources for Salt Stress Tolerance Genes and Promoters." Frontiers in Plant Science, vol. 8, 2017, doi:10.3389/fpls.2017.00829.

seeds were in sealed bags and the supplier stated that the seeds were untreated. The supply of NaCl could also limit consistency as purity could be different. While purchasing, specifically asking for pure NaCl specifically and if purchasing online, looking for the purity percentage, which is usually noted, is important.

Further Investigation

After evaluating the experiment and looking at other articles about similar experiments, I found areas in my experiment that can be investigated in further experiments for improvement. As I mentioned in the evaluations part, many halophytes have many seed species which have been stated to be salt tolerant. Therefore, after this experiment, I would like to experiment with other halophyte species in order to examine their salt tolerance and their efficiency for usage with the increase in soil salinity.

Furthermore, soil includes many different salts, as have been mentioned in evaluations, and I have only investigated with NaCl. In order to get a better understanding of salinity's effect on seed germination, experimenting with multiple salt types would be effective for further investigation. I would also like to experiment with germination in saline soil with different seed species to directly examine the saline soil's effect instead of water-salt solutions.

Bibliography

1. Shannon, Michael C. “Adaptation of Plants to Salinity.” Advances in Agronomy, 1997, pp. 75–120., doi:10.1016/s0065-2113(08)60601-x.
2. Shrivastava, Pooja, and Rajesh Kumar. “Soil Salinity: A Serious Environmental Issue and Plant Growth Promoting Bacteria as One of the Tools for Its Alleviation.” Saudi Journal of Biological Sciences, vol. 22, no. 2, 2015, pp. 123–131., doi:10.1016/j.sjbs.2014.12.001.
3. Jamil, A., et al. “Gene Expression Profiling of Plants under Salt Stress.” Critical Reviews in Plant Sciences, vol. 30, no. 5, 2011, pp. 435–458., doi:10.1080/07352689.2011.605739.
4. Greenway, H, and R Munns. “Mechanisms of Salt Tolerance in Nonhalophytes.” Annual Review of Plant Physiology, vol. 31, no. 1, 1980, pp. 149–190., doi:10.1146/annurev.pp.31.060180.001053.
5. Flowers, Timothy J., and Timothy D. Colmer. “Salinity Tolerance in Halophytes*.” New Phytologist, vol. 179, no. 4, 2008, pp. 945–963., doi:10.1111/j.1469-8137.2008.02531.x.
6. Raven, Peter H., et al. Biology of Plants. W.H. Freeman, 1987. pp. 504–508.
7. HARDEGREE, STUART P., and WILLIAM E. EMMERICH. “Partitioning Water Potential and Specific Salt Effects on Seed Germination of Four Grasses.” Annals of Botany, vol. 66, no. 5, 1990, pp. 587–595., doi:10.1093/oxfordjournals.aob.a088068.
8. “Seed Germination and Seedling Growth of Suaeda Salsa under Salt Stress.” Annales Botanici Fennici, vol. 44, no. 3, Finnish Zoological and Botanical Publishing Board, 2007, pp. 161–69, http://www.jstor.org/stable/23727638.
9. Flowers, T. J., et al. “Halophytes.” The Quarterly Review of Biology, vol. 61, no. 3, 1986, pp. 313–337., doi:10.1086/415032.

10. Yamamoto, Kosuke, et al. “Molecular Cloning Ofacetylcholinesterasegene Fromsalicornia Europaeal.” Plant Signaling & Behavior, vol. 4, no. 5, 2009, pp. 361–366., doi:10.4161/psb.4.5.8360.

11. Mer, R. K., et al. “Effect of Salts on Germination of Seeds and Growth of Young Plants of Hordeum Vulgare, Triticum Aestivum, Cicer Arietinum and Brassica Juncea.” Journal of Agronomy and Crop Science, vol. 185, no. 4, 2000, pp. 209–217., doi:10.1046/j.1439-037x.2000.00423.x.

12. Iizumi, Toshichika, and Toru Sakai. “The Global Dataset of Historical Yields for Major Crops 1981–2016.” Scientific Data, vol. 7, no. 1, 2020, doi:10.1038/s41597-020-0433-7.

13. Norton, Barbara J., and Michael J Strube. “Guide for the Interpretation of Two-Way Analysis of Variance.” Physical Therapy, vol. 66, no. 3, 1986, pp. 402–412., doi:10.1093/ptj/66.3.402.

14. Miyamoto, S., et al. Photo Guide: Landscape Plant Response to Salinity. Agricultural Research and Extension Center at El Paso, Texas Agricultural Experiment Station, the Texas A & M University System, 2004.

15. Tran, Dan Q., et al. “Ion Accumulation and Expression of Ion Homeostasis-Related Genes Associated with Halophilism, NaCl-Promoted Growth in a Halophyte Mesembryanthemum Crystallinum L.” Plant Production Science, vol. 23, no. 1, 2019, pp. 91–102., doi:10.1080/1343943x.2019.1647788.

16. Calone, Roberta, et al. “Assessing Salicornia Europaea Tolerance to Salinity at Seed Germination Stage.” Agriculture, vol. 10, no. 2, 2020, p. 29., doi:10.3390/agriculture10020029.

17. ÖNER, Fatih, and Ayşegül KIRLI. "Effects of Salt Stress on Germination and Seedling Growth of Different Bread Wheat (Triticum Aestivum L.) Cultivars." Akademik Ziraat Dergisi, 2018, pp. 191–196., doi:10.29278/azd.476365.
18. Shrivastava, Pooja, and Rajesh Kumar. "Soil Salinity: A Serious Environmental Issue and Plant Growth Promoting Bacteria as One of the Tools for Its Alleviation." Saudi Journal of Biological Sciences, vol. 22, no. 2, 2015, pp. 123–131., doi:10.1016/j.sjbs.2014.12.001.
19. Mishra, Avinash, and Bhakti Tanna. "Halophytes: Potential Resources for Salt Stress Tolerance Genes and Promoters." Frontiers in Plant Science, vol. 8, 2017, doi:10.3389/fpls.2017.00829.

Appendix

Calculations for Concentration Uncertainty

The percentage uncertainty of Mol will be equal to percentage uncertainty of Mass of NaCl as no other measurement was used. It will be kept in percentage uncertainty in data table as it will be more useful for the calculation of uncertainty of concentration.

Sample calculation for percentage uncertainty for 0.02 mol of NaCl which was measured as 1.17g

$$\frac{Absolute\ uncertainity}{measurement}\ x\ 100$$

$$\frac{0.01}{1.17}\ x\ 100$$

$$\approx \pm \%\ 0.9\ (1\ \text{S.F. due to}\ 0.01)$$

Sample calculation for uncertainty of 0.100 mol L^{-1}

$$\%\ uncertainity\ water\ volume = \frac{0.0001}{0.2000} = \%\ 5\ x\ 10^{-4}$$

As the uncertainty of water volume is to small, the only measurement that will have a significant uncertainty is Mols of NaCl. Therefore %uncertainty of NaCl mols will be equal to that of molarity of Solution.

$$Absolute\ uncertainity\ 0f\ 0.100\ mol\ L^{-1}$$

$$= 0.100\ x\ 0.009$$

$$= \pm 9\ x\ 10^{-4}\ mol\ L^{-1}$$

The data from concentration of solution have 3 decimal places, rounded according to smallest significant figure of the measurement of molarity; the absolute uncertainty calculated will be too small to be significant therefore it won’t be considered.