

What is the effect of *Nickel (II) Chloride* concentration on the germination rate (%) of *Triticum aestivum* seeds?

Introduction

With the ongoing industrialization of the world, abiotic stress factors due to urbanization have led to the incorporation of pollutants such as pesticides, household chemical waste products, and heavy metals in natural resources such as soil and water, degrading not only the environmental quality but entire ecosystems as a whole. Soil is a valuable and non-renewable resource essential for the growth of plants, thus supporting the majority of ecosystems in the world. Agriculture is something all human life is dependent upon. Early cities were built around rivers, as they provided the most fertile soil that would lead to an increase in crop yield. The presence of various by-products of human activity, such as mining, can have detrimental impacts on organic life through bioaccumulation. Growing up surrounded by the effects of the growing climate crisis, the implications of human activities on the environment has been a field of investigation that I have been interested in for quite a while. Researching the effect of pollution on crops came to me when my mother told me about how she read an article that said that the food we eat today is nearly ten times less nutrient-dense than the food that people ate a decade ago. These figures aren't very scientifically accurate but reading about how different factors such as the distance between the crops and the nearest urban region can impact the nutrient quality and growth of plants inspired me to conduct this experiment.

Investigation

1. Background information:

Bioaccumulation can be defined as the net accumulation of contaminants in organisms, relative to that in the environment. It is resultant of all uptake and loss processes, including respiration and dietary uptake, egestion, and metabolism¹. The presence of heavy metals, such as nickel, mercury, and copper in air, soil and water result in bioaccumulation, which ends up affecting the entire ecosystem, as plants act as primary producers. The ecological impacts that the bioaccumulation of toxins present include damage to vegetation, soils composition, the inhibition of revegetation, and the disruption of aquatic ecosystems.

The major sources of heavy metal ion pollution are the overburdening of mines, and the usage of industrial effluent, fertilizers and pesticides. Metal ions are toxic to the metabolic processes of plants, mainly through their interaction with the functional groups of molecules in the cell, especially proteins and polynucleotides. A high concentration of metal ions in soil has been attributed to reduced shoot and root elongation, oxidative damage, membrane alterations, and nutrient loss. Germination is the resumption of active growth of the embryonic axis in a seed.

¹ Borgå, K. "Ecotoxicology: Bioaccumulation." Reference Module in Earth Systems and Environmental Sciences, 2013, doi:10.1016/b978-0-12-409548-9.00765-x. Accessed 15th May 2020.

This is indicated by the rupture of the encasing and a visible plant root and is essential to the propagation of a plant.

Nickel has been shown to be highly toxic to most plant species, as it affects the enzymatic activity of various essential enzymes, such as amylase, protease, and ribonuclease thus has been shown to cause retardation of seed germination and growth of many crops². Furthermore, the presence of nickel ions has been attributed to reducing plant height, root length, chlorophyll content³. Nickel stress has also been reported to cause the accumulation of electrolyte ions in plant stores and impact photosynthetic machinery.⁴ *Triticum aestivum* or common wheat is the most widely grown of all crops and is the cereal with the greatest monetary yield. It has been widely used in clinal phytotoxicity studies as it is rapidly growing and is relatively easy to analyse.

2. Aim:

To investigate the effects of varying concentrations of Nickel (II) Chloride (0, 30, 60, 90, 120 mg/L) on the germination rate of *Triticum aestivum* by observing the number of seeds germinated over a period of 5 days in solutions of 0-5 days.

3. Hypothesis:

Alternate hypothesis (H_1): As nickel ion concentration increases, the germination rate of *Triticum aestivum* decreases. This is as a higher nickel ion concentration leads to a greater interference in the activity of essential ribonuclease and protease enzymes, primarily due to an accumulation of electrolyte ions in seed stores. Additionally, the bioaccumulation of nickel is associated with a decline in membrane stability and the mobilisation and digestion of food reserves in germinating seeds.

Null hypothesis (H_0): As nickel ion concentration increases, there is no change in the germination rate of *Triticum aestivum*.

4. Experimental variables:

Independent variable: Nickel (Ni^+) ion concentration (ppm) ($\pm 0.01\text{ppm}$)
Solutions of 0, 30, 60, 90, 120 mg/L were used.

Hydrous nickel (II) chloride was used, as this is the most common soluble form of nickel. These values were chosen in accordance with the nickel ion concentration found in Indian rivers, from

² Baruah, Nijara, et al. "Influence of Heavy Metals on Seed Germination and Seedling Growth of Wheat, Pea, and Tomato." *Water, Air, & Soil Pollution*, vol. 230, no. 12, 2019, doi:10.1007/s11270-019-4329-0. Accessed 15th May 2020.

³ Chen, Cuiyun, et al. "Functions and Toxicity of Nickel in Plants: Recent Advances and Future Prospects." *CLEAN - Soil, Air, Water*, vol. 37, no. 4-5, 2009, pp. 304–313., doi:10.1002/clen.200800199. Accessed 20th May 2020.

⁴ Ghosh, Shyamasree, and Sunilkumar Sethy. "Effect of Heavy Metals on Germination of Seeds." *Journal of Natural Science, Biology and Medicine*, vol. 4, no. 2, 2013, p. 272., doi:10.4103/0976-9668.116964. Accessed 16th May 2020.

a report published by the Central Water Commission⁵. Concentrations from 0 to 120 mg/L further allowed for the investigation to explore the effect of varying levels of nickel ion presence. A concentration of 0 mg/L provides a control group, which can be used as a point of comparison for the other groups. The control group values were also used in the calculation of the germination rate.

Dependent variable: The number of seeds successfully germinating over a period of 5 days.

This was used to calculate the germination rate of the *Triticum aestivum* samples. This was chosen as the germination rate is used as a measure of the time course of seed germination and allows us to effectively compare the rates of each batch of seeds. This was calculated using the following germination rate formula

$$\text{Germination rate} = \frac{\% \text{ Control group germination} - \% \text{ Tested group germination}}{\% \text{ Control group germination}}$$

Controlled variables:

Variable	Reasoning	Method of control
Amount of water	The germination of seeds is dependent on the availability of ample amounts of water.	100ml of water was measured and provided to the initial seed set-up. The same amount (10 ml) of water was additionally added every day.
Source of water	Waters from differing sources have different electrolyte ion concentration and will differ in their hardness, which has been shown to impact seed germination. ⁶ Distilled water lacks additional minerals and electrolytes that could interfere with nickel activity.	Distilled water was used from a singular source to prevent disparity. In addition to this, the same distilled water used for the control was used to make the NiCl ₂ solutions.
Temperature	The temperature can impact seed germination by affecting metabolic enzyme activities. 25° C was chosen as it has been shown to be the ideal temperature for <i>T. aestivum</i> germination ⁷ .	Seeds were kept at a constant temperature of 25° C by conducting the experiment in a closed room with an air conditioner.
Light available	The amount of available light can impact the germination of seeds	All seedlings were kept in the same area, resulting in no variation between groups.

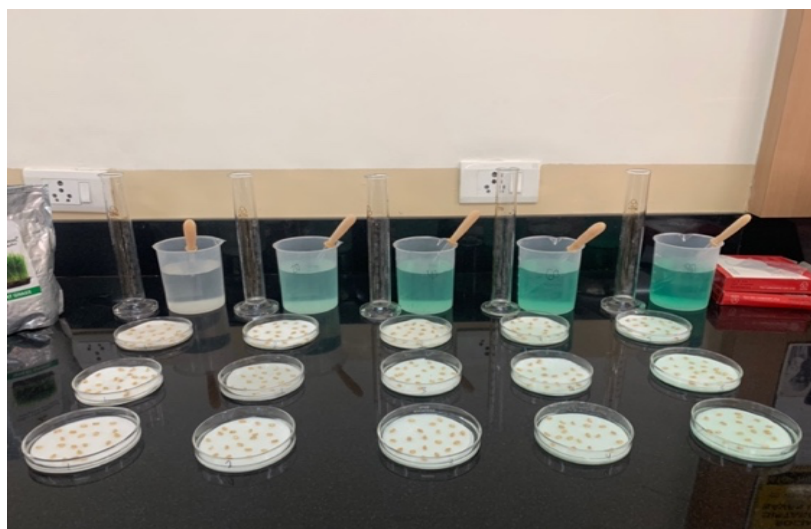
⁵ River Data Directorate, Ministry of Water Resources, Government of India, 2014, Status of Trace and Toxic Metals in Indian Rivers - 2014. Accessed 12th August 2020.

⁶ Maity, Moumita, et al. "Water Quality Demonstrates Detrimental Effects on Two Different Seeds during Germination." *Current Agriculture Research Journal*, vol. 7, no. 3, 2019, pp. 377–389., doi:10.12944/carj.7.3.14. Accessed 12th August 2020.

⁷ Ceccaroni, Dayana, et al. "Effect of the Time and Temperature of Germination on the Phenolic Compounds of *Triticum Aestivum*, L. and *Panicum Miliaceum*, L." *LWT*, vol. 127, 2020, p. 109396., doi:10.1016/j.lwt.2020.109396. Accessed 12th August 2020.

Source of seeds	Different sources of <i>T. aestivum</i> seeds will possess differing genetic characteristics which may impact the rate of germination.	All seeds were sourced from the same plant supply store, minimizing this disparity.
Quality of seeds	Some seeds may be damaged or have a lower mass, which may impact their germination rate.	The seeds were visually examined, and any seeds that appeared to be discoloured, damaged, or abnormally small were removed.

5. Pilot study: Before conducting the final experiment, a pilot study was conducted with Nickel (II) chloride solutions of 80 mg/L, 120 mg/L, 160mg/L, and 210 mg/L in order to ascertain the value at which germination activity was entirely inhibited. This was found to be the solution at 160 mg/L; thus 120 mg/L was chosen as the maximum solution concentration.



A self-taken image showing the setup for the pilot study

Methodology

1. Materials:

- Glassware:

Name of apparatus	Volume (ml)	Least count (ml)	Uncertainties (if used for measurement) (ml)	Number used in one trial
Petri dishes	-	-	-	10
250ml graduated cylinder	250	2	± 0.400	5
1l beaker	1000	-	-	5
50ml graduated cylinder	50	5	± 0.100	5

- Apparatus:
 - Graduated pipette (1)
 - 10.0 cm ruler (± 0.050 mm)
 - Filter paper
 - Electric mass balance (± 0.001 g)
 - Stirring rod (1)

- Chemical and biological materials:
 - *Triticum aestivum* seeds (360 seeds)
 - Nickel (II) Chloride (840 mg)
 - Distilled water (15 litres)

2. Procedure:

Preparation of seeds and beakers

- a) Using the ruler, measure the length of the *Triticum aestivum* seeds and pick 10 seeds around 0.7cm in width.
- b) Leave all seeds in a petri dish filled with distilled water overnight.
- c) Fill 6 1l beakers with distilled water up to the 1l marked line
- d) Label the beakers for each of the concentrations required (0, 30, 60, 90, 120 mg/L)

Preparation of solutions

The following steps e) to g) were repeated for each NiCl₂ solution.

- e) Weigh 30 mg of anhydrous NiCl₂ using the mass balance.
- f) Transfer the weighed powder to its cognate 1l beaker.
- g) Using a stirring rod, stir the solution until no visible sediment can be seen.

Preparation of Petri dishes

- h) Label 5 Petri dishes from A to E, corresponding to all 5 concentrations.
- i) Measure 25ml of each NiCl₂ solution. Pour these into labelled 50ml beakers numbered from 1-5.
- j) In a petri dish, cut filter paper to size and layer 4 sheets of filter paper to provide a base for seed germination.
- k) Pour the corresponding solution into each petri dish. Using a stirring rod, press down the sheets so that the entire surface is saturated with the NiCl₂ solution.
- l) Place 10 *Triticum aestivum* seeds on the surface of each petri dish.
- m) Place the 5 dishes in a sunny area close to each other.

Data collection

- n) Wait for 5 days. Each day add 10ml of the corresponding solution to each petri dish.
- o) Count the number of seeds with a visible shoot longer than 10mm in length.
- p) Repeat the experiment thrice with each concentration to improve the reliability of data.

The seeds were observed for a period of a week, to allow for sufficient time to gauge the effectiveness of each solution of varying Ni⁺ ion concentration on the growth rate of the seedlings. Since the Petri dishes were placed next to each other, and trials were conducted simultaneously, they are assumed to be receiving equal amounts of light. One method of data collection for this experiment was to count the number of seeds that germinated successfully. This was done by observing whether the seed coat of each seed was broken and whether the seedling had emerged. The statistical significance between Ni⁺ ion concentration and seed germination were found using the χ^2 test.

3. Risk assessment:

Safety considerations: Nickel (II) chloride is toxic if ingested and can be a respiratory/dermatological irritant, thus the experiment was conducted using latex gloves and a lab coat to ensure safety.

Environmental considerations: Nickel (II) chloride has long term adverse impacts on aquatic ecosystems; thus, it was not emptied down a sink and was disposed of following hazardous waste protocols.

Ethical considerations: There were no ethical considerations to take into account.

Raw Data

1) Qualitative Data:

- The solutions with a higher concentration of nickel (II) chloride had a slight blue tint.
- The seed coats of seeds in the 0 and 30 mg/L solutions showing signs of germination as soon as the second day.
- The seeds in the 0 mg/L solution were already showing steady shoot growth at the end of the week.
- The shoots of the seeds in the 90 and 120mg/L groups showed minor irregularities in form compared to those in the 0 mg/L groups.
- The seeds in the higher concentration groups appeared to take on the blue tint of the nickel (II) chloride solutions by day 4.

2) Quantitative Data:

Data Table 1: A raw data table containing the number of seeds germinated in each trial

Concentration of Nickel ions (mg/L)	Number of seeds germinated (/20)					
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
0	19	20	20	21	19	20
30	16	19	17	16	18	16
60	14	16	14	15	13	14
90	13	11	10	12	11	12
120	9	12	10	10	8	9

Data Processing

Table 1 shows an inverse correlation between the concentration of the NiCl₂ solution and the number of germinated seeds. In order to better understand this relationship and coherently display the collected data, the mean of the values from each trial will be determined. Furthermore, the standard deviation of these values will be determined in order to ascertain the dispersion within the dataset.

The average number of seeds germinated was calculated using the following formula:

$$\frac{\text{Sum of seeds germinated in each trial}}{120} = \text{Average number of seeds germinated}$$

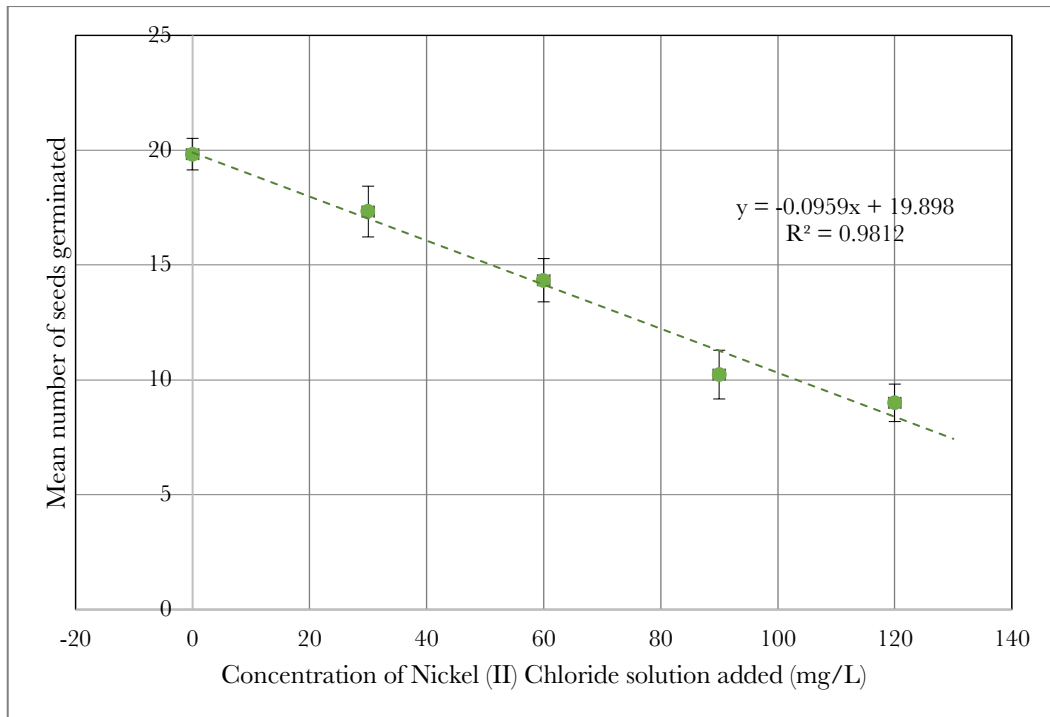
The standard deviation of values was calculated using the following formula:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{120} (x_i - \bar{X})^2}{120}}$$

Data Table 2: A processed data table containing the averages and standard deviation of the number of seeds germinated at each nickel ion concentration.

Concentration of Nickel ions (mg/L)	Average number of seeds germinated (0/20)	Standard deviation of values
0	19.83	0.687
30	17.33	1.105
60	14.34	0.942
90	10.23	1.061
120	9.01	0.816

Graph 1: A graph showing the average number of seeds germinated at each nickel ion concentration showing the standard deviation through error bars



This graph, in addition to the data depicted in Table 2, supports the alternative hypothesis, as they showcase a negative correlation with the concentration of Nickel ions. To better represent the effects of Nickel ion concentration on seed germination, the germination rate will be calculated.

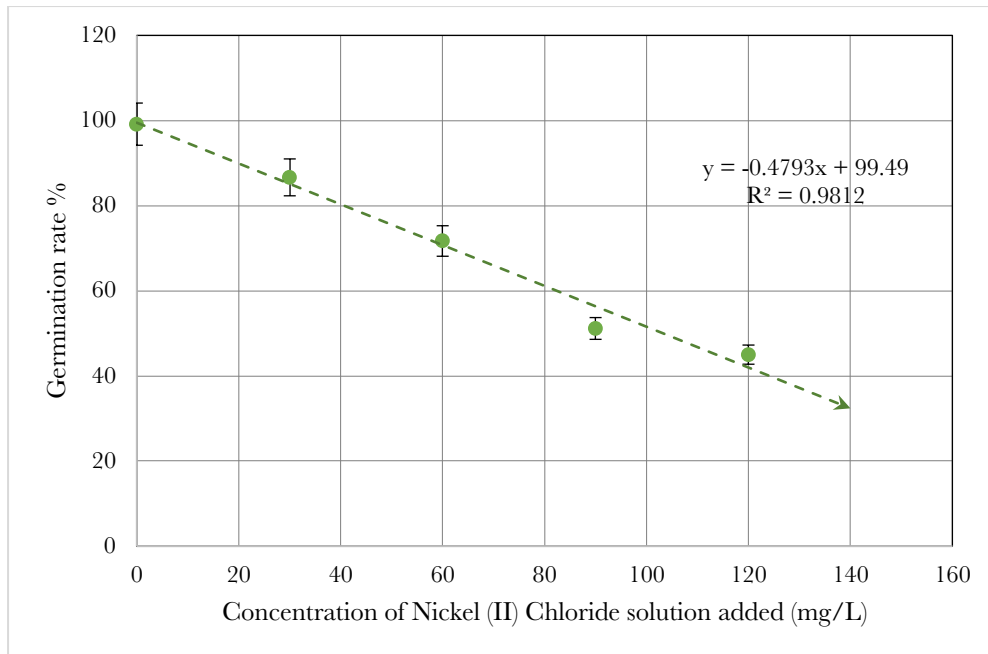
$$\text{Germination rate} = \frac{\text{Number of seeds germinated}}{\text{Total seeds}}$$

Data Table 3: A processed data table containing the calculated germination rate of *Triticum aestivum* seeds at each nickel ion concentration

Concentration of Nickel ions (mg/L)	Average number of seeds germinated	Germination rate (%)
0	19.83	99.15
30	17.33	86.65
60	14.34	71.70
90	10.23	51.15
120	9.00	45.00

The calculated germination rates further support the alternative hypothesis, in order to better depict the relationship between nickel ion concentration and germination rate, a scatter graph is plotted, with error bars at a standard value of 5%.

Graph 2: A graph of the germination rate of *Triticum aestivum* seeds at each nickel ion concentration



To ascertain the variability of the data collected, the percentage error derived from the equipment utilized will be calculated.

Margin of error in the mass balance: ± 0.001 g

Margin of error in the 250 ml graduated cylinder: ± 0.5 ml

Margin of error in the 50 ml graduated cylinder: ± 0.4 ml

25ml of each NiCl_2 was measured using the 50ml graduated cylinder, thus the percentage uncertainty for the same would be the following:

$$\frac{0.4}{25} \times 100 = 1.60 \%$$

The mass balance used to weigh the NiCl_2 required to make each solution had a margin of error of ± 0.001 g. The smaller the measured value, the higher the uncertainty. Substituting the minimum measured mass (30mg) into the same we get the following:

$$\frac{0.001}{0.03} \times 100 = 3.33\%$$

The 250ml graduated cylinder was used to adjust the NiCl_2 solutions to the final volume of 1 litre. The 250ml graduated cylinder has a margin of error of ± 0.5 ml.

$$\frac{0.5}{250} \times 100 = 0.2 \%$$

Adding together all the uncertainties, we get the maximum percentage uncertainty (%) of 5.12%

Data Analysis

A maximum percentage uncertainty of 5.12%, as well as the standard deviation values, suggest an acceptable level of variance and uncertainty within the dataset. The R_2 value of both Graph 1 and 2 is 0.981. The R_2 value is a measure of how well a linear regression model fits a dataset. These values range from 0 to 1. A value of 0.98 indicates a strong relationship between the model and the dependant variable. Graph 2, as well as the difference in percentage germination, falls in line with the alternative hypothesis, showing a negative correlation between nickel (II) chloride concentration and percentage germination, however, to test the statistical significance of this relationship, the ANOVA test was conducted.

The following working hypotheses were used in the conduction of the ANOVA test:

H_0 : There is no difference in the means of the five concentration groups.

H_1 : There is a significant difference in the means of the five concentration groups.

Table 10 (From Microsoft Excel): *A table displaying the input and results of the ANOVA test.*

<u>Anova Test: Single Factor</u>						
SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
0 mg/L	6	118	19.666667	0.26666667		
30 mg/L	6	104	17.333333	1.46666667		
60 mg/L	6	86	14.333333	1.06666667		
90 mg/L	6	73	12.166667	1.36666667		
120 mg/L	6	54	9	0.8		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	422.6667	4	105.66667	106.375839	2.57306E-15	2.75871
Within Groups	24.83333	25	0.9933333			
Total	447.5	29				

The p-value was found to be below the accepted significance limit of 0.05, which shows that the differences in the averages of the five sets of values are similar, further, a significance level below <0.001 suggests that the relationship between the variables are highly statistically significant. Thus, the null hypothesis can be rejected and the alternative hypothesis, stating that there is a significant difference between the means of NiCl_2 concentration and the germination of *Triticum aestivum* seeds.

Evaluation

Table 11: A table displaying possible sources of errors and improvements

Error	Type of error	Significance	Method of Improvement
Process used to determine germination of seeds	Systematic error	The criteria of a visible shoot longer than 10mm in length were used as a standard in order to ensure accurate data.	Germination could have also been monitored through enzyme activity as a more accurate means of determining germination activity.
Substrate utilised	Systematic error	Cotton was used as a substrate for the germinating seeds. Soil and treated potting mixtures contain essential nutrients that assist in seed germination.	Usage of a treated potting mixture for germinating seeds.
Usage of distilled water in the solution	Systematic error	The NiCl ₂ solutions were created using distilled water, which lacks essential minerals which play a role in regulating hydrolytic enzyme activity ⁸ . However, the usage of tap water may have impacted the activity of nickel ions and would have been difficult to standardise.	Bottled drinking water could have been used in order to have a source of essential minerals at a controlled concentration.
Measurement of the <i>Triticum aestivum</i> seed size	Systematic error	The approximate diameter of the seeds was measured with a ruler prior to usage in the study, which is not a very accurate method of determining the viability of the seeds utilised.	The mass of the seeds could have been measured in order to reduce any disparity between the seeds.
Control of external variables	Random error	Germination is reliant on light availability and enzyme activity, which is sensitive to temperature. All samples were placed in an area receiving the same amount of natural light and ventilation at a controlled temperature, the experimental conditions could have been further controlled.	The experiment could have been conducted in a room with more ventilation and a better temperature control system. Furthermore, lamps could have been used as a light source in order to reduce any disparity due to light availability.
Sources of data	Limitation	This paper only utilised primary data as a source of evaluation. Although the data acquired is proved to be of statistical value, the usage of secondary data could have assisted in understanding the relationship between nickel ion concentration and seed germination.	Secondary data taken from databases showcasing regional germination rates or crop yields could have been compared with soil data gathered from agricultural surveys in order to ascertain whether the same correlation is present in an in-vivo setting.
Frame of observation	Limitation	As discussed in the Background Research section, nickel stress has been attributed to the reduction of plant height, root length, and chlorophyll content. Studying these variables would also allow us to gain a deeper understanding of the impact of nickel stress on long-term plant welfare, instead of just seed germination.	Studying the growth of the seeds over the period of 2-3 weeks instead of a singular week.

⁸ Ali, Awatif S., and Alaaeldin A. Elozeiri. "Metabolic Processes During Seed Germination." *Advances in Seed Biology*, 2017, doi:10.5772/intechopen.70653. Accessed 16th August 2020.

Conclusion

The experiment shows that nickel stress can dramatically impact *Triticum aestivum* seed germination rates. The germination rate observed for the control was 99.15%, whereas the rate observed for the highest nickel concentration used (120mg/L) was 45.00%. Although some improvements could have been made to improve the quality of the data obtained, an assessment of the measures of uncertainty and variance, as well as the ANOVA test show that the data is of statistical significance, allowing us to reject the null hypothesis and accept the alternative hypothesis. As illustrated in graph 2, the relationship follows an inverse correlation, with an increase in NiCl₂ concentration resulting in a decreased germination rate, in line with the alternative hypothesis presented. The standard deviation values are shown in Table 2 suggest a low level of random error in the experiment, which evidences the accuracy of the data collected. The maximum percentage uncertainty was found to be 5.12%, which is just over the value of 5% typically accepted within scientific research. This can be attributed to the low level of precision of the mass balance used. The high R² value of graph 2 indicates a strong inverse correlation between the concentration of NiCl₂ in solution and the germination of *Triticum aestivum* seeds. This can be attributed to the toxicological effects that nickel ions have on the enzyme activity and photosynthetic machinery required for seed germination. Nickel stress has also been found to affect the digestion and mobilisation of energy reserves in germinating seeds, and the membrane stability of growing plants. Furthermore, the bioaccumulation of nickel ions has been known to impact the activity of ribonuclease and protease enzymes, retarding seed germination and causing a decline in growth, membrane stability, and lipid peroxidation⁴. This can be seen in the data collected since the rate of germination of seeds in the 0 mg/L groups was 54.15% higher than that of seeds in the 120 mg/L group. This additionally explains why the shoots emerging from the seeds in the 60-120 mg/L groups showed slight irregularities in form.

Further Scope: Nickel is an essential nutrient for plants, as it plays a role in regulating tissue urea levels and antioxidant metabolism⁹, however understanding its impact as a phytotoxin as a side effect of mine exhaustion and industrial pollution is important to consider in a time of great ecological strain, where many countries are facing devastation due to agricultural issues and a lack of nutrients. Broadening the scope of the experiment, studying the impact of various heavy metal salts on seed germination, would be a means of delving deeper into the interactions of these ions with seeds, allowing us to better understand how researchers can manipulate pathways to prevent impact on agricultural produce as well as human health in the long-term in order to combat the strain put on agricultural sectors by the reduction in soil quality.

⁹ Fabiano, Caio C., et al. "Essentiality of Nickel in Plants: a Role in Plant Stresses." *Frontiers in Plant Science*, vol. 6, 2015, doi:10.3389/fpls.2015.00754. Accessed 16th August 2020.

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