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MAKALE BİLGİSİ / ARTICLE INFORMATION

Geliş Tarihi / Date Received
20.01.2024

Kabul Tarihi / Date Accepted
25.11.2024

Yayın Tarihi / Date Published
Aralık / December 2024

Yayın Sezonu / Pub Date Season
Aralık - Haziran / December - June

ATIF / CITE as

Akbaş, Ç. (2024).“ Cu+, Mn+, ve Ni+ İyonlarının Farklı Konsantrasyonlarının *Glycine max* (Soya Fasulyesi) Çimlenmesi Üzerindeki Etkileri” /“ Effects of Different Concentrations of Cu+, Mn+, And Ni+ Ions on *Glycine max* Germination”. Bilar: Bilim Armonisi Dergisi, 7 (2): 26-36 doi. 10.37215/bilar.1422890

https://dergipark.org.tr/tr/pub/bilar

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Özet

Bu çalışma, sülfat tuzları içindeki Cu+, Mn+ ve Ni+ iyonlarının değişen konsantrasyonlarının soya fasulyesi (*Glycine max*) tohumlarının çimlenme ve büyümesine olan etkisini araştırmaktadır. Yüksek sayıda endüstri komplekslerinin bulunduğu bölgelerdeki tarım arazileri yerel gıda kaynaklarına ve sağlığa risk oluşturur. Endüstri merkezlerinden gelen, Cu, Ni, Pb ve Cr gibi ağır metallerle kirlenen atık sular tarım alanlarını kirletebilir, bitkileri ve tohumları etkileyebilir. Bu çalışma, *Glycine max* tohumlarının farklı konsantrasyonlardaki (0.2 M, 0.1 M ve 0.05 M) $CuSO_4$, $MnSO_4$ ve $NiSO_4$ tuzlarına maruz bırakıldığındaki çimlenmeleri ve büyümeleri üzerindeki etkilerini araştırmaktadır. Çalışma, çözelti türleri ve konsantrasyonlarının tohum büyümesi üzerindeki etkisininin istatiksel olarak değerlendirmek için iki yönlü ANOVA ve regresyon analizleri kullanmaktadır. Sonuçlar, artan ağır metal konsantrasyonları ile tohum büyümesi arasında güçlü bir negatif korelasyon olduğunu göstermektedir. Ancak, farklı metal iyonlarının etkileri arasında istatiksel olarak anlamlı bir fark gözlemlenmemiştir. Çalışma, Cu^+, Mn^+ ve Ni^+ iyonlarının sülfat tuzlarındaki artan konsantrasyonlarının tohum büyümesinde önemli bir azalmaya neden olduğunu göstermektedir. Bulgular, tarım uygulamalarında ağır metal kontaminasyonunun potansiyel risklerini vurgulamakta ve absorbe edilen ağır metallerin bitkilerden insan sağlığına transferi üzerine daha fazla araştırmaya gerek olduğunu ortaya sunar.

Anahtar Kelimeler: soya fasulyesi, *Glycine max*, tohum, büyüme, çimlenme, bakır sülfat, nikel sülfat, ağır metaller, $CuSO_4$, $MnSO_4$, $NiSO_4$, mangan sülfat

ABSTRACT

This study investigates the impact of varying concentrations of Cu^+, Mn^+, and Ni^+ ions in sulfate salts on the germination and seed growth of soybeans (*Glycine max*). In regions with a high density of industrial complexes, agricultural lands often coexist with industrial activities, posing a risk to local food supplies and health. Wastewater from industrial centers, rich in heavy metals such as Cu, Ni, Pb, and Cr, can contaminate agricultural areas, affecting crops and seeds. This study explores the germination and growth of *Glycine max* seeds exposed to different concentrations (0.2 M, 0.1 M, and 0.05 M) of $CuSO_4$, $MnSO_4$, and $NiSO_4$ salts. The study employs two-way ANOVA and regression analyses to assess the significance of solution types and concentrations on seed growth. The results indicate a strong negative correlation between increasing heavy metal concentrations and seed growth. However, no significant difference is observed among the effects of different metal ions. The study concludes that increased concentrations of Cu^+, Mn^+, and Ni^+ ions in sulfate salts lead to a significant decrease in seed growth. The findings emphasize the potential risks of heavy metal contamination in agricultural practices and call for further research on the transfer of absorbed heavy metals in crops to human health. Presented at Bilim Armonisi International Youth Congress on December 15th, 2023.

Keywords: soybeans, *Glycine max*, seed, growth, germination, copper sulfate, manganese sulfate, nickel sulfate, heavy metals, $CuSO_4$, $MnSO_4$, $NiSO_4$

The study was presented as an oral presentation at the 2023 Science Harmony International Youth Congress.

1. INTRODUCTION

1.1. Research Question

What effects do different concentrations of Cu^{+}, Mn^{+}, and Ni^{+} ions have on soybean (*Glycine max*) germination and seed growth?

1.2. Context

In general city planning, cities consist of economic, residential, agricultural, and industrial centers. Most of the time, industrial and agricultural centers are located next to each other. This causes some basic issues. Studying at a boarding school located in one of the most industrialized centers in the region, the negative aspects of living next to an immense industrial activity drew my attention. The main issue is the fact that the surroundings of these industrial areas are mostly used for agricultural practices. A disruption in these farmlands results in greater problems in the local food supply and health. Considering that our food is supplied from the local farmer's markets, the school population is most likely subjected to those negative effects. Likewise, crops and seeds that are irrigated with contaminated waters may display disruptions in growth, resulting in economic damage.

With the advancements in technology, the rate of industrial production increases, and the number of industrial complexes significantly rises (Sethy and Ghosh 2013). Chemical-intensive industries produce great amounts of wastewater. Because of the chemical processes involving heavy metals, the wastewater from industrial centers contains high concentrations of heavy metals such as Cu, Ni, Pb, and Cr, implying an environmental health hazard. Since salts of heavy metals are highly soluble in water, the biological community that is dependent on the local water resources that are next to industrial complexes can easily absorb the dissolved heavy metals. Considering the food chain and the biomagnification effect, which can be defined as the process in which the substances become more concentrated at the next trophic level, humans and animals are at risk of serious health problems such as cancer, organ damage, irreversible nervous system damage, immune defense failure, intrauterine growth retardation, psychosocial dysfunction, malnutrition, and gastrointestinal neoplasms (Barakat 2012, Geng et al. 2020). To prevent these negative effects, wastewater treatment techniques are being advanced and more popular.

This investigation will seek to display the effects of irrigation with contaminated water on plants. Heavy metals intoxicate crops and cause a decrease in crop yields, germination, and growth (Sethy and Ghosh 2013).

1.3. Background

1.3.1. Heavy Metal Deposition

Heavy metals are defined as naturally occurring elements that have higher atomic masses and at least 5 times greater density than that of water (Tchounwou et al. 2014). Two groups of activities result in significant environmental deposition of heavy metals: Anthropogenic and natural.

One of the most significant human-derived sources of contaminated wastewater is industries that are involved in electroplating and metal surface treatment. They are responsible for the deposition of substantial amounts of heavy metals such as Zn, Pb, Cu, Pt, Cd, and Ni to the environment, mostly via wastewater. Likewise, printed circuit board production is also a significant source of heavy metals in wastewater such as Sn. Moreover, petrochemical industries and oil refineries – for example, the one that is only a few kilometers away from our school district – contribute to a high Ni and Cr contamination in the surrounding environment. Therefore, we can conclude that regions with a greater number of industrial complexes and centers that process heavy metals are more contaminated with heavy metals through wastewater.

Even though the most probable way of heavy metal deposition is through human-derived causes, some natural processes are also involved in increasing heavy metal concentrations in an environment. Volcanic eruptions and lava release heavy metals that are trapped deeper in the Earth's crust. Also, the weathering of rocks increases the likelihood of the dissolution of ions of heavy metals in water and later leaching into greater water bodies, later leading to groundwater contamination (Nwaichi et al. 2014). The ones that can not leach through the groundwater, accumulate on the surface (Aydinalp and Marinova 2009).

In a nutshell, the soil can have a metal concentration range between less than 1mg/kg and as high as 100000mg/kg due to both natural and anthropogenic causes (Aydinalp and Marinova 2009). Higher concentrations of various heavy metals such as Nickel, Copper, and Zinc in the soil have displayed a great role in ecological imbalances, even though these species are essential as micronutrients in plants' survival (Aydinalp and Marinova 2009).

Nickel (Ni) is considered highly toxic for the majority of plant species. Ni can act as an inhibitor in several metabolic pathways. Sethy and Ghosh (2013) suggest that Ni disrupts the structure of several enzymes such as amylase, protease, and ribonuclease enzyme. Ribonuclease enzyme is essential in protein synthesis and cell division. Amylase and protease are responsible

for indigestion and indispensable for providing nutrients to the body. Therefore, by affecting key enzymes in an organism, higher concentrations of Ni inhibit growth. In plants, the germination process and fertility are also negatively affected by Ni, as it prevents digestion of carbohydrates and proteins, reducing catalytic activity and developing oxidative stress. Observations include shorter stem height, root weight, lower mass, and reduced chlorophyll concentration (Sethy and Ghosh 2013).

Resembling Ni's properties, copper (Cu) is also essential and beneficial for metabolic processes. Positively contributing to blood formation, carbohydrate digestion, collagen formation, and keratin amounts, Cu displays toxicity as well. The reduction process of Cu(II) to Cu(I) generates superoxide and hydroxyl radicals (Tchounwou et al. 2014). Therefore, Cu may lead to a reduced germination rate in plants and cause toxicity. Cu stress in plants inhibits the digestion of starch and sucrose since it leads to the induction of glucose and fructose release and disrupts the activities of alpha-amylase and invertase (Sethy and Ghosh 2013).

Manganese (Mn) is an essential micronutrient with both beneficial and potentially harmful effects on plant growth. As a critical cofactor, Mn supports several enzymatic reactions, including those involved in photosynthesis, nitrogen metabolism, and carbohydrate synthesis. It is indispensable for the function of the oxygen-evolving complex in photosystem II, enabling water-splitting and oxygen evolution during photosynthesis. However, Mn toxicity occurs in waterlogged or acidic soils, where excessive Mn availability generates oxidative stress, disrupts root growth, and inhibits nutrient uptake. Mn deficiency similarly affects plant health, causing reduced chlorophyll concentration, stunted growth, and impaired photosynthetic efficiency (Schmidt and Husted 2019, Alejandro et al. 2020).

Intensive wastewater treatment techniques and centers need to be developed to minimize the concentrations of heavy metals in wastewater to protect the environment from the negative effects of the growing industries as "Human activities such as industrial and agricultural production, and transportation increase Cu, Zn and Cd enrichment factors" (Guo et al. 2019, Barakat 2012)

1.3.2. Relevance of This Investigation

Ajiboye et al. (2021) point out that using wastewater for agricultural irrigation purposes has become popular among African farmers as wastewaters provide a high amount of nutrients. Moreover, farmers who use wastewater as an irrigation source seem to earn a greater income than those who do not use wastewater income (Ajiboye et al. 2021). Therefore, we can claim that using wastewater attracts farmers as a primary irrigation source. However, as mentioned earlier, wastewater contains a higher level of heavy metal concentrations than tap water. Irrigating crops with wastewater for an extended period results in accumulating heavy metal particles in the soil. For example, Ajiboye et al. (2021) deduced mean concentrations of Cu, Mn, and Ni ions in the sample soil as 1.12 mg/L, 0.84 mg/L, and 0.13 mg/L respectively (Ajiboye et al. 2021). As heavy metals are considered dangerous for plants due to their toxic traits, increased heavy metal concentration and accumulation in the soil will damage crop yield by inhibiting key metabolic processes that affect germination, growth, and reproduction in plants and seeds (Sethy and Ghosh 2013). Most studies naturally focus on the negative effects on human health. However, this investigation questions whether specific heavy metal solutions with different concentrations affect growth in soybean seeds.

1.3.3. Preliminary Testing

Cu displayed a greater effect on seed germination when tested against other heavy metals in five different concentrations (Baruah et al. 2019).

Observations concluded that plants that are exposed to greater than 50 mg/kg concentrations of Ni suffered from toxicity and displayed symptoms accordingly; yet, very low concentrations seemed to contribute to the synthesis of the urease enzyme (Aydinalp and Marinova 2009).

To collect results in a shorter time, *Glycine max* (soybean) seeds are considered for the investigation. *Glycine max* is estimated to germinate approximately after two days. Also, testing the effects of heavy metals on *Glycine max* germination is essential since soybean is located among the greatest sources of vegetable oil and livestock feed. Moreover, *Glycine max* has a 40-42% of protein content, having the most percentage of protein content compared to other crops. Likewise, *Glycine max* highly contributes to the nitrogen fixation process when sowed in farmland. Therefore, *Glycine max* draws significant importance in global food supply and agricultural production as a protein source and a significant actor in nitrogen fixation (Pagano and Miransari 2016).

A study on *Eruca sativa* germination suggested the use of a maximum of 1 mM for each heavy metal solution. However, only Ni displayed a significant difference in germination and growth with this concentration (Zhi et al. 2015). Therefore, using three concentrations of Ni, Cu, and Mn ions ranging between 0.2 M and 0.05 M was decided.

1.3.4. Hypotheses

Hypothesis 1:

Given the higher toxicity levels and role as an inhibitor in several metabolic processes, Ni^+ displays the greatest inhibitory effect on *Glycine max* germination than Cu^+ and Mn^+.

Hypothesis 2:

Higher concentrations of Cu^+, Ni^+, and Mn^+ will demonstrate a greater inhibitory effect on *Glycine max* growth than lower concentrations.

2. METHODOLOGY

2.1. Rationale

The two-way ANOVA test aims to analyze if there is a significant difference between the effects of different sulfate solutions on seed growth. In this examination, seed growth is calculated by the average percentage change in seed mass. Regression analyses aim to display the correlations between the solution concentrations and growth. A greater R^2 value will display a strong correlation. These analyses are essential for testing the significance of the results and validating the hypotheses.

To investigate the effects of different metal ions on seed growth, aqueous solutions of metal salts should be considered. In the literary survey, most of the experiments used chloride salts. However, due to the unavailability of chloride salts in the school's chemistry laboratory, it was decided to use sulfate salts of Cu, Mn, and Ni. Sulfate ions are considered harmless and less insignificant than heavy metals in terms of acting as a growth inhibitor. In this investigation, growth will be determined through a germination process. *Glycine max* seeds are used due to their quick germination.

2.2. Variables

2.2.1. Independent Variables

A total of two independent variables will be assessed in this investigation, solution type, and concentration. The tested solutions consist of aqueous solutions of $CuSO_4$, $MnSO_4$, and $NiSO_4$ salts. Likewise, different concentrations are tested as 0.2 M, 0.1 M, and 0.05 M. Because water is the solvent for these salts, the controlled group included the same tap water that was used in the salt solutions as the control group.

2.2.2. Dependent Variables

In this experiment, the growth which is determined via seed mass and percentage change in seed mass in grams (g) is the dependent variable. This is measured using a lab balance (±0.01g).

2.2.3. Controlled Variables

The total days of the experiment are kept constant for each seed sample (total of 6 days), as growth is measured after 6 days for each seed.

Each seed is selected with an initial mass of 0.19g from the same package of the same brand; the mass is measured with the same scale. Seed sterilization was not conducted. Seeds that displayed visual anomalies such as odd color, darkness, and wrinkles on the seed coat were eliminated to maintain the quality and health of each seed.

Germination conditions are kept constant for each sample. Temperature and oxygen amount in the air is important in germination since the temperature is essential to initiate germination and the germinating seeds need oxygen for respiration. They are all kept in the same medium, and exposed to the same temperature until the last day of the experiment. However, on day 2, all the samples were transferred to another medium where the temperature was kept constant throughout the day. This will be discussed later in the Evaluation of the Method.

All samples including the control group are watered with the same amount of solutions. On day 0, all the samples are watered with 10mL of responding solutions. Then, on Day 3, they are watered with 10mL of solutions.

Also, the seeds are germinated in Petri dishes of the same size and volume. The amount of cotton used for the sample preparation is kept constant via eyeball estimate, yet additional cotton was added when needed for water absorption throughout the experimental process.

Moreover, samples are kept in a box to prevent light. Therefore, the growth by the photosynthesis factor is eliminated. Thus, measured growth will express only the effects of the mentioned independent variables.

Finally, since the metal-to-sulfate ratio is 1:1 in all salt solution samples, the amount of SO_{4-} ions was the same in different solution groups. Therefore, the differences due to the possible effects of sulfate ions on growth are eliminated.

2.3. Materials

Measurement Equipment	General Apparatus	Chemicals
(1) electronic weighing scale ±0.01g	(30) *G. max* seeds	1000 mL of 0.2 M $CuSO_4$ solution
(1) 10 mL graduated cylinder ± 0.1 mL	(30) 100mm x 15mm sterilized polystyrene Petri dishes	1000 mL of 0.2 M $MnSO_4$ solution
(1) 5mL graduated pipette ±0.1 mL		1000 mL of 0.2 M $NiSO_4$ solution
(1) 5mL volumetric pipette ±0.1 mL	(1) forceps	1000 mL of tap water
(1) 1000 mL graduated cylinder ± 5mL	(3) spatula	
	(1) magnetic stirrer	
	(2) 500 mL erlenmeyer flask	
	(2) funnel	
	(1) marker	
	(15) pairs of latex gloves	
	(3) tweezers forceps	
	(1) safety googles	
	(1) lab coat	
	(2) 250 mL beaker	
	(1) 1000 mL beaker	
	(1) 1000 mL florence flask	

Table 1: quantities of materials required for the experiment

Materials are provided from the school's biology and chemistry laboratories. While using

the equipment, biology, and chemistry teachers were consulted.

2.4. Experimental Procedure

2.4.1. Experimental Preparations

In the first step of the experimental process, the solutions are prepared. For convenience, the 0.2 M solutions are prepared, so that it would be easier to produce the smaller concentrations later while watering the samples. Hydrated salts are obtained from the school's chemistry lab. Atomic masses for $CuSO_4 \cdot 5H_2O$, $MnSO_4 \cdot H_2O$, and $NiSO_4 \cdot 6H_2O$ are accepted as 249.709 amu, 169.02 amu, and 262.87 amu respectively; 49.95 g, 33.80 g, and 52.57 g of respective hydrated salts are used to prepare the 0.2 M solutions. Salts are poured into 1 L florence flasks after being weighed on the balance. Then flasks are made up to 1 L by pouring tap water from the same source. $NiSO_4$ solution immediately dissolved in water while $CuSO_4$ needed a magnetic stirrer to dissolve. Solutions are prepared in 1 L due to assuming that the seeds would need to be watered every day with 15 mL of solutions.

The flasks are tagged and a warning sign is put on the flask with $NiSO_4$ solution. During the preparation of chemicals, latex gloves, goggles, and a lab coat are used while also safety data sheets in Appendices 1-3 are considered.

The second step involved selecting seeds and preparing the Petri dishes. Seeds that weighed 19 mg were selected for the experiment. Cotton pieces from a brand-new package were placed as two layers in Petri dishes; seeds were put in between.

2.4.2. Germination

On day 0, each sample was watered with 10 mL of their respective solutions and concentrations. 15 mL of solutions were not used after observing a peer's experiment in which 15 mL of solutions were not absorbed by the cotton sheets in Petri dishes. Sulfate solutions were poured with caution due to their high toxicities. 0.1 M and 0.05 M concentrations of the solutions are prepared right before being poured on the seeds. For 0.1 M solutions, 5 mL of the 0.2 M solution and 5 mL of tap water were measured in a graduated cylinder and mixed in a beaker; then the new solution with a concentration of 0.1 M was poured on the seeds. The same process is followed for preparing 0.05 M solutions: 2.5 mL of the 0.2 M solution and 7.5 mL of tap water are mixed. In the end, the Petri dishes were placed in a box.

The same process is applied on day 3. Unlike the assumption made while preparing the 0.2 M solutions, no additional solution was added on the other days of the experiment since the cotton sheets were still wet until the end of day 2.

2.4.3. Collecting Results

Starting from day 1, the weights of the seeds are measured with an electronic weighing scale once a day for 5 days. Each seed had a total of 5 readings. The results are noted and form the raw data at the end of the experiment.

To prevent contact with the toxic solutions, each seed was carried with tweezer forceps. After, collecting results, Petri dishes were placed in the closed box.

2.5. Risk Assessment

The chemicals used in this experiment are considered dangerous. Especially in the preparation part, the dust of the sulfate salts should not be breathed directly. Direct contact with solutions is avoided and materials are thoroughly rinsed after being used. A lab coat was worn during the preparation and data collection processes. Hands were immediately washed after contacting solutions. All the waste produced during the experiment was placed in the contaminated waste container in the lab building. For further precautions, protocols in safety data sheets in Appendices 1-3 are referred to.

3. DATA COLLECTION AND PROCESSING

3.1. Raw Data

See Appendices 4-7

3.2. Processed Data

3.2.1. Qualitative Data

$CuSO_4$ Solution									
Molarity	0.20 M			0.10 M			0.05 M		
Observations	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Radicle formation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Germination	No	No	No	No	No	No	No	No	No

$MnSO_4$ Solution									
Molarity	0.20 M			0.10 M			0.05 M		
Observations	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Radicle formation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Germination	No	No	Yes	No	Yes	Yes	No	Yes	Yes

$NiSO_4$ Solution									
Molarity	0.20 M			0.10 M			0.05 M		
Observations	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Radicle formation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Germination	No	No	No	No	No	No	No	No	No

Tap Water			
Observations	Trial 1	Trial 2	Trial 3
Radicle formation	Yes	Yes	Yes
Germination	No	No	No

Table 2: Qualitative observations after Day 5

Germination and radicle formation are observed as qualitative data. According to Table 1, every seed displayed radicle formation. Radicle formation includes the visually observable radicles within the seed coat, even when there is no germination. However, only the seeds that were watered with tap water and $MnSO_4$ solutions germinated. Also, black spots formed on the seeds that were watered with $MnSO_4$ solutions.

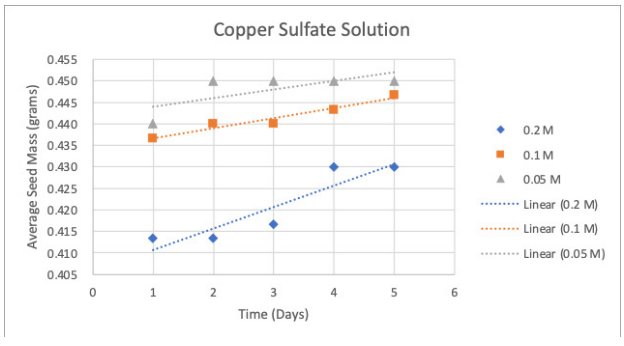
$CuSO_4$ and $NiSO_4$ solutions left blue and green stains on the seeds, respectively.

3.2.2. Quantitative Data

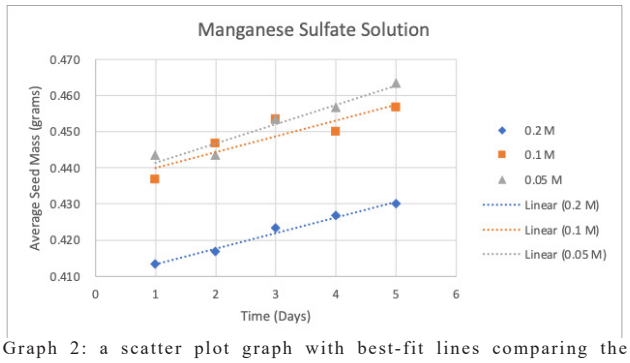
Average Seed Mass ((± 0,01g)										
Molarity	$CuSO_4$ Solution			$MnSO_4$ Solution			$NiSO_4$ Solution			Tap Water
Time (Days)	0.20 M	0.10 M	0.05 M	0.20 M	0.10 M	0.05 M	0.20 M	0.10 M	0.05 M	Control Group
0	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190
1	0.413	0.413	0.413	0.413	0.437	0.443	0.407	0.420	0.430	0.420
2	0.413	0.413	0.413	0.417	0.447	0.443	0.407	0.427	0.443	0.427
3	0.417	0.417	0.417	0.423	0.453	0.453	0.410	0.433	0.447	0.480
4	0.430	0.430	0.430	0.427	0.450	0.457	0.407	0.437	0.447	0.507
5	0.430	0.430	0.430	0.430	0.457	0.463	0.413	0.440	0.447	0.557

Table 3: Average seed mass of different groups for each day

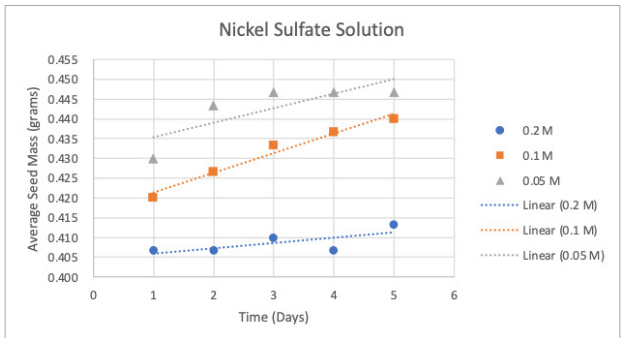
After collecting data from the three trial groups for each solution type with different concentrations, Table 3 was developed by averaging those raw data. The mass data for Trial 1 of 0.05 M $CuSO_4$ solution is not used for calculating the average and its data was eliminated since it did not show any growth or absorbed water.



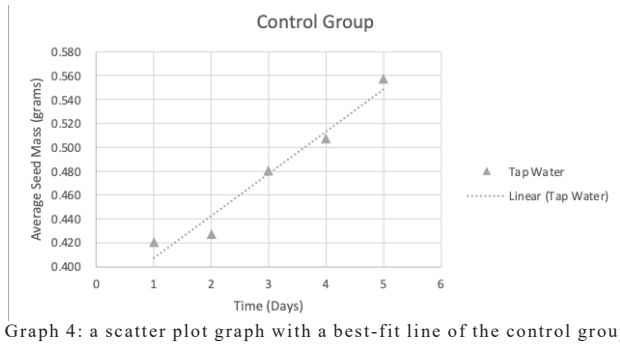
Graph 1: a scatter plot graph with best-fit lines comparing the effects of different concentrations of $CuSO_4$ on average seed mass



Graph 2: a scatter plot graph with best-fit lines comparing the effects of different concentrations of $MnSO_4$ on average seed mass

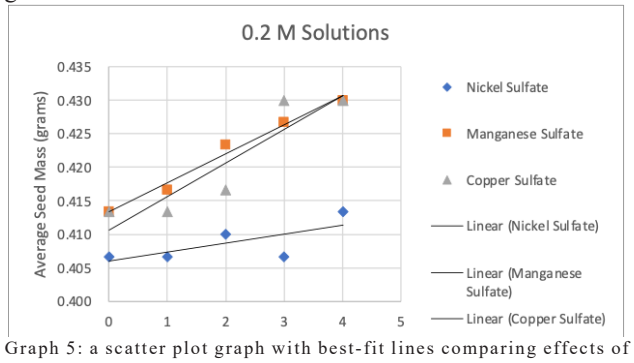


Graph 3: a scatter plot graph with best-fit lines comparing the effects of different concentrations of $NiSO_4$ on average seed mass

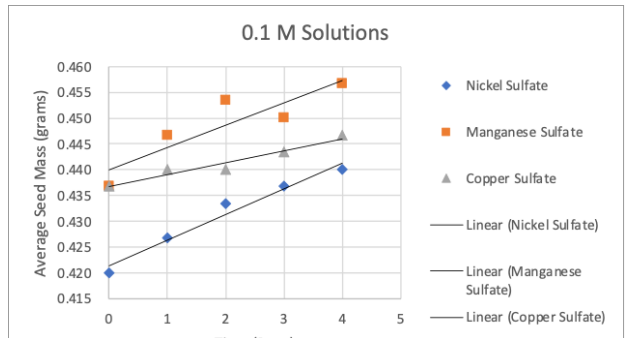


Graph 4: a scatter plot graph with a best-fit line of the control group on average seed mass

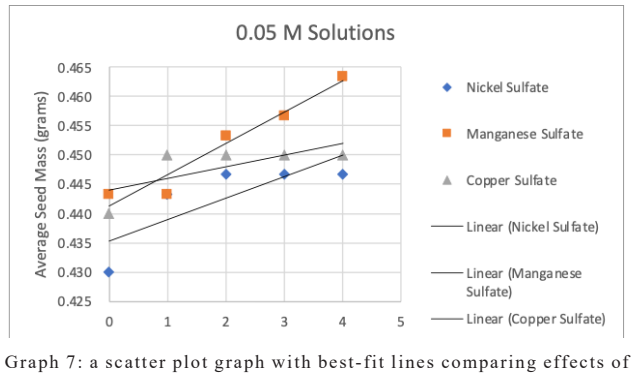
Graphs 1, 2, and 3 display the average seed mass values for different concentrations of $CuSO_4$, $MnSO_4$, and $NiSO_4$ solutions for each day of the experiment. According to the graphs, higher concentrations resulted in less average seed mass. Also, each concentration group displayed an increase in average seed mass values throughout the experiment. The control groups displayed average seed mass values between 0.420 – 0.560 grams.



Graph 5: a scatter plot graph with best-fit lines comparing effects of 0.2 M of heavy metal solutions on average seed mass



Graph 6: a scatter plot graph with best-fit lines comparing effects of 0.1 M of heavy metal solutions on average seed mass



Graph 7: a scatter plot graph with best-fit lines comparing effects of 0.05 M of heavy metal solutions on average seed mass

Graphs 5, 6, and 7 display the average seed mass values for different solution types of 0.2 M, 0.1 M, and 0.05 M concentrations for each day of the experiment. According to the graphs, $NiSO_4$ solutions resulted in less average seed mass. Also, each solution group displayed an increase in average seed mass values throughout the experiment. The maximum average seed mass value is observed in 0.05 M $MnSO_4$ solution (excluding the values from the control group), while 0.2 M $NiSO_4$ solution shows the minimum average seed mass value.

3.3. Statistical Analysis

3.3.1. Regression Analysis and One-Way ANOVA

	Average % change in seed mass		
Molarity	Copper Sulfate	Manganese Sulfate	Nickel Sulfate
0.05	242.105	243.860	235.088
0.1	235.088	240.351	231.579
0.2	226.316	226.316	217.544

Table 4: Average percentage changes in seed mass

Average percentage change values were generated for collected seed mass values regarding the independent variables. These values will be referred to as growth values. To analyze the correlation between increasing concentrations of each solution type and growth, a regression analysis was conducted and R^2 values for each solution type were generated. The regression analyses were conducted on Office Excel software. The analyses also ran one-way ANOVA and tested the significance of the difference between the effects of different concentration groups on seed growth. Therefore, the following hypotheses were tested.

Hypotheses
Null Hypothesis (H_0): There is no difference in the means of the different concentrations.
Alternate Hypothesis (H_a): The means of the different concentrations are not all equal

Table 5: hypotheses for one-way ANOVA

Manganese Sulfate Regression Analysis Data					
Regression Statistics					
Multiple R	0.989743319				
R Square	0.979591837				
Adjusted R Square	0.959183673				
Standard Error	1.875517487				
Observations	3				
ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	168.8431605	168.8431605	48	0.091257897
Residual	1	3.517565844	3.517565844		
Total	2	172.3607264			

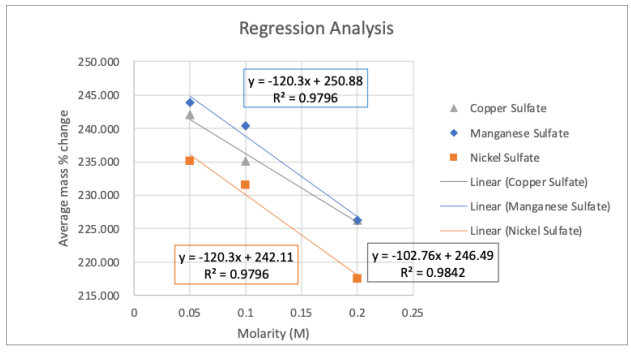
Table 6: Regression Analysis Data For $MnSO_4$

Copper Sulfate ANOVA and Regression Analysis Data					
Regression Statistics					
Multiple R	0.992064533				
R Square	0.984192037				
Adjusted R Square	0.968384075				
Standard Error	1.406638115				
Observations	3				
one-way ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	123.1880872	123.1880872	62.25925926	0.080254424

Table 7: Regression Analysis Data For $CuSO_4$

Nickel Sulfate Regression Analysis Data					
Regression Statistics					
Multiple R	0.989743319				
R Square	0.979591837				
Adjusted R Square	0.959183673				
Standard Error	1.875517487				
Observations	3				
ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	168.8431605	168.8431605	48	0.091257897
Residual	1	3.517565844	3.517565844		
Total	2	172.3607264			

Table 8: Regression Analysis Data For $NiSO_4$



Graph 8: Regression Analysis of Glycine max grown in different molarities of Sample Solutions for 6 days with linear trend lines

As observed in Graph 8 and Tables 6, 7, and 8, R^2 values for $CuSO_4$, MnSO4, and $NiSO_4$ are estimated as 0.9842, 0.9796, and 0.9796 respectively. Also, one-way ANOVA results from Tables 6, 7, and 8 for each solution displayed F>Significance F. Therefore, the alternate hypothesis (Ha) on Table 5 is accepted for all solution types.

3.3.2. Two-Way ANOVA

Groups	Copper Sulfate	Manganese Sulfate	Nickel Sulfate
	236.842	215.789	200.000
	200.000	210.526	231.579
0.2 M	242.105	252.632	221.053
	242.105	242.105	231.579
	231.579	247.368	210.526
0.1 M	231.579	231.579	252.632
	231.579	242.105	252.632
	242.105	252.632	210.526
0.05 M	236.842	226.316	242.105

Table 9: Average percentage change in seed mass of independent variables

A two-way ANOVA was conducted on Office Excel for the values in Table 9. A two-way ANOVA evaluates the significance of two independent variables on dependent variables altogether. Hypotheses for two-way ANOVA are displayed in Table 10.

Hypothesis	Explanation
INTERACTION	
Null Hypothesis (H_0)	"There is no interaction between factors A and B"
Alternate Hypothesis (H_a)	"There is an interaction between factors A and B"
INDEPENDENT VARIABLE A: SOLUTION TYPE	
Null Hypothesis (H_0)	"There is no difference in the means of independent variable A"
Alternate Hypothesis (H_a)	"There is at least one difference in the means of independent variable A; not all means are equal"
INDEPENDENT VARIABLE B: CONCENTRATIONS	
Null Hypothesis (H_0)	"There is no difference in the means of independent variable B"
Alternate Hypothesis (H_a)	"There is at least one difference in the means of independent variable B; not all means are equal"

Table 10: hypotheses for two-way ANOVA

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1052.63158	2	526.31579	1.865454549	0.183548775	3.554557146
Columns	264.6968307	2	132.3484153	0.469090911	0.633006267	3.554557146
Interaction	49.24592178	4	12.31148044	0.043636364	0.996056794	2.927744173
Within	5078.485684	18	282.1380936			
Total	6445.060016	26				

Table 11: results of two-way ANOVA

Results of two-way ANOVA displays P-value > α for each independent variable. Therefore, null hypotheses (H_0) were accepted in Table 13.

Hypothesis	Conclusion
INTERACTION	
Null Hypothesis (H_0)	reject H_a and accept H_0 as the p-value (0.9961) > α-value (0.050)
Alternate Hypothesis (H_a)	
INDEPENDENT VARIABLE A: SOLUTION TYPE	
Null Hypothesis (H_0)	reject H_a and accept H_0 as the p-value (0.6330) > α-value (0.050)
Alternate Hypothesis (H_a)	
INDEPENDENT VARIABLE B: CONCENTRATIONS	
Null Hypothesis (H_0)	reject H_a and accept H_0 as the p-value (0.1835) > α-value (0.050)
Alternate Hypothesis (H_a)	

Table 12: hypothesis testing from the two-way ANOVA

4. DISCUSSION AND EVALUATION

4.1. Evaluation of Results

Graph 8 and regression testings suggested strong correlations between increased average percentage change of seed mass and decreased concentrations of different solution types because of the high R^2 values. For all three solutions, increased concentrations suggested a decrease in seed growth. Also, one-way ANOVA testing for each solution type suggested the significance of the correlation between concentrations and seed growth within the same solution groups. Therefore, it can be concluded that there is a strong correlation between increasing concentrations of heavy metal salts and seed growth. Thus, Hypothesis 2 is accepted (higher concentrations of Cu^+, Ni^+, and Mn^+ will demonstrate a greater inhibitory effect on *Glycine max* growth than lower concentrations).

However, in the two-way ANOVA, the null hypotheses were accepted due to high p-values. This result suggests that even though the different concentrations have a significant correlation with the growth, different solution types do not significantly decrease or increase growth. However, in Graph 8, one can observe that the inhibitory effects of solutions can be ordered as:

$$NiSO_4 > CuSO_4 > MnSO_4$$

Yet, this observational result remains insignificant considering the two-way ANOVA. Therefore, Hypotheses 1 was not accepted (Given the higher toxicity levels and role as an inhibitor in several metabolic processes, Ni^+ displays the greatest inhibitory effect on *Glycine max* germination than Cu^+ and Mn^+).

Also, only the seeds that were watered with $MnSO_4$ solutions were germinated among those watered with a sulfate salt solution. The germination rates for 0.2 M, 0.1 M, and 0.05 M $MnSO_4$ solutions are respectively 1/3, 2/3, and 2/3. Even though any concentration of $CuSO_4$ and $NiSO_4$ solutions did not display germination, radicle formation within the seed coat was observed for each sample. Therefore, growth occurred, but in a very small amount.

Higher R^2 values from the regression tests also suggest the consistency of the collected data. Finally, the investigation concludes that regardless of solution types, used heavy metal salts display a strong correlation between increasing growth and decreasing concentration levels.

4.2. Evaluation of the Methodology

Even though growth was observed in this experiment, instead of cof llecting seed data, stem or seedling lengths could be evaluated as an indicator of growth. However, because relatively higher levels of concentrations of sulfate salt solutions are used in this experiment to observe results quickly (which was revealed to be a not good assumption), the overall germination rate was quite lower. Likewise, two-way ANOVA would benefit from fewer concentration groups and more days.

The first flaw of the method was revealed after realizing that pouring samples with 15 mL was excessive considering the cotton sheets' absorption. Also, on day 1 the samples did not need watering and were still wet. Therefore, the solutions were poured a total of two times.

The thickness of cell walls or the metabolic pathways within the germinating seeds could be measured as a growth indicator via microscope, since heavy metals inhibit key metabolic processes (Sethy and Ghosh 2013).

Finally, the first plan of the experiment was based on using chloride salts of heavy metals since the majority of studies that were encountered during the preliminary research used chloride ions.

However, as a limitation, the school's chemistry lab did not have chloride salts of heavy metals. Therefore, sulfate salts are used for the experiment. Sulfate ions are considered harmful on plants only in extreme concentrations. Moreover, since all solutions contained sulfate ions, it was a controlled variable, the comparison of differences in heavy metal ions was not affected.

5. CONCLUSION

Ultimately, the research question was answered by this investigation. In conclusion, 0.05 M of solutions demonstrated the highest seed growth, and 0.2 M of concentrations demonstrated the smallest seed growth. Thus, it is concluded that as the concentrations of $NiSO_4$, $CuSO_4$, and $MnSO_4$ increase, the level of seed growth decreases.

However, no significant difference among the effects of different salt types was found while observations from Graphs 5, 6, and 7 suggested there could be a significant correlation. Thus, it is concluded that different salt types do not affect seed growth differently.

Moreover, the effects of increased heavy metal concentrations are considered inhibitory on seed germination. Therefore, agriculture might suffer from the dissolved heavy metals in the irrigation water. Since this investigation focused on the effects of heavy metals on plants, more investigations can be conducted on what percentage of the absorbed heavy metals in crops can settle in the human body as a relevant and serious research topic in a heavily industrialized world.

6. LITERATURE

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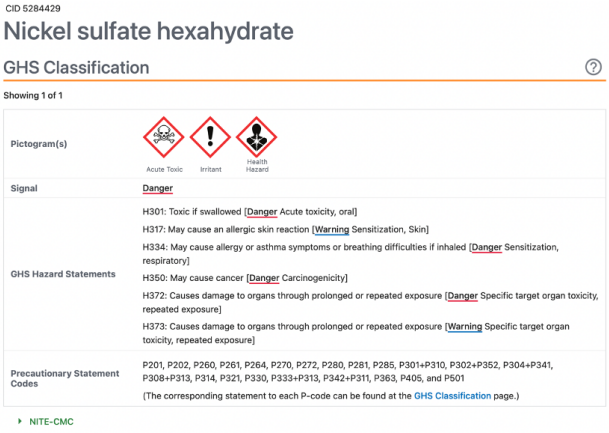
7. APPENDICES



CID 5284429

Nickel sulfate hexahydrate

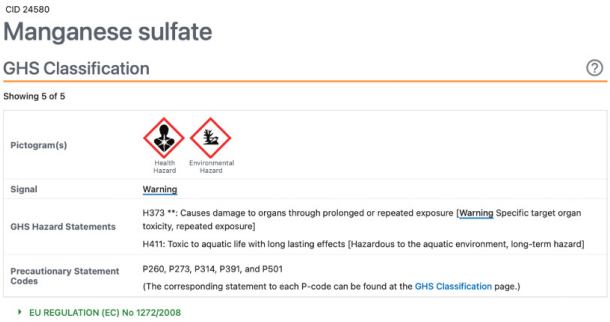
GHS Classification

Showing 1 of 1

Pictogram(s)	Acute Toxic, Irritant, Health Hazard
Signal	Danger
GHS Hazard Statements	H301: Toxic if swallowed [Danger Acute toxicity, oral] H317: May cause an allergic skin reaction [Warning Sensitization, Skin] H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled [Danger Sensitization, respiratory] H350: May cause cancer [Danger Carcinogenicity] H372: Causes damage to organs through prolonged or repeated exposure [Danger Specific target organ toxicity, repeated exposure] H373: Causes damage to organs through prolonged or repeated exposure [Warning Specific target organ toxicity, repeated exposure]
Precautionary Statement Codes	P201, P202, P260, P261, P264, P270, P272, P280, P281, P285, P301+P310, P302+P352, P304+P341, P308+P313, P314, P321, P330, P333+P313, P342+P311, P363, P405, and P501 (The corresponding statement to each P-code can be found at the GHS Classification page.)

NITE-CMC

Appendix 1: *National Center for Biotechnology Information (2022). PubChem Compound LCSS for CID 5284429, Nickel sulfate hexahydrate. Retrieved March 9, 2022 from https://pubchem.ncbi.nlm.nih.gov/compound/Nickel-sulfate-hexahydrate#datasheet=LCSS.*



CID 24580

Manganese sulfate

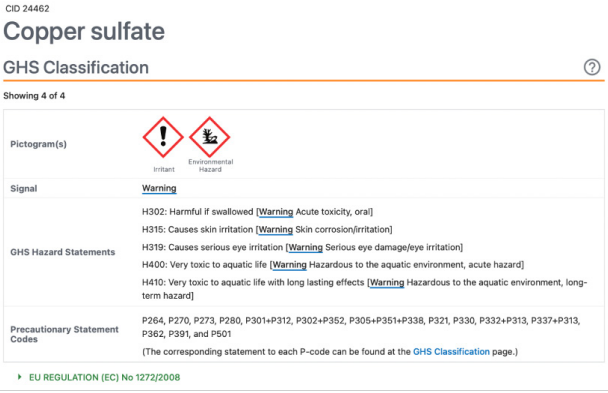
GHS Classification

Showing 5 of 5

Pictogram(s)	Health Hazard, Environmental Hazard
Signal	Warning
GHS Hazard Statements	H373 **: Causes damage to organs through prolonged or repeated exposure [Warning Specific target organ toxicity, repeated exposure] H411: Toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment, long-term hazard]
Precautionary Statement Codes	P260, P273, P314, P391, and P501 (The corresponding statement to each P-code can be found at the GHS Classification page.)

EU REGULATION (EC) No 1272/2008

Appendix 2: *National Center for Biotechnology Information (2022). PubChem Compound LCSS for CID 24580, Manganese sulfate. Retrieved March 9, 2022 from https://pubchem.ncbi.nlm.nih.gov/compound/Manganese-sulfate#datasheet=LCSS.*



CID 24462

Copper sulfate

GHS Classification

Showing 4 of 4

Pictogram(s)	Irritant, Environmental Hazard
Signal	Warning
GHS Hazard Statements	H302: Harmful if swallowed [Warning Acute toxicity, oral] H315: Causes skin irritation [Warning Skin corrosion/irritation] H319: Causes serious eye irritation [Warning Serious eye damage/eye irritation] H400: Very toxic to aquatic life [Warning Hazardous to the aquatic environment, acute hazard] H410: Very toxic to aquatic life with long lasting effects [Warning Hazardous to the aquatic environment, long-term hazard]
Precautionary Statement Codes	P264, P270, P273, P280, P301+P312, P302+P352, P305+P351+P338, P321, P330, P332+P313, P337+P313, P362, P391, and P501 (The corresponding statement to each P-code can be found at the GHS Classification page.)

EU REGULATION (EC) No 1272/2008

Appendix 3: *National Center for Biotechnology Information (2022). PubChem Compound LCSS for CID 24462, Copper sulfate. Retrieved March 9, 2022 from https://pubchem.ncbi.nlm.nih.gov/compound/Copper-sulfate#datasheet=LCSS.*

$CuSO_4$ Solution									
	Seed Mass (± 0.01g)								
Molarity	0.20 M			0.10 M			0.05 M		
Time (Days)	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190
1	0.420	0.360	0.460	0.480	0.430	0.400	0.190	0.420	0.460
2	0.430	0.360	0.450	0.460	0.440	0.420	0.190	0.440	0.460
3	0.430	0.370	0.450	0.470	0.430	0.420	0.190	0.440	0.460
4	0.450	0.380	0.460	0.460	0.430	0.440	0.190	0.440	0.460
5	0.450	0.380	0.460	0.460	0.440	0.440	0.410	0.440	0.460

Appendix 4*: Raw Data Collection for $CuSO_4$ Solutions*

$MnSO_4$ Solution									
	Seed Mass (± 0.01g)								
Molarity	0.20 M			0.10 M			0.05 M		
Time (Days)	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190
1	0.400	0.390	0.450	0.440	0.460	0.410	0.450	0.450	0.430
2	0.400	0.380	0.470	0.450	0.460	0.430	0.450	0.450	0.430
3	0.400	0.390	0.480	0.460	0.470	0.430	0.460	0.470	0.430
4	0.410	0.390	0.480	0.460	0.460	0.430	0.460	0.470	0.440
5	0.410	0.400	0.480	0.460	0.470	0.440	0.460	0.480	0.450

Appendix 5*: Raw Data Collection for MnSO4 Solutions*

$NiSO_4$ Solution									
	Seed Mass (± 0.01g)								
Molarity	0.20 M			0.10 M			0.05 M		
Time (Days)	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190
1	0.370	0.430	0.420	0.450	0.380	0.430	0.460	0.400	0.430
2	0.360	0.430	0.430	0.430	0.400	0.450	0.480	0.400	0.450
3	0.370	0.440	0.420	0.430	0.400	0.470	0.480	0.400	0.460
4	0.360	0.440	0.420	0.440	0.400	0.470	0.480	0.400	0.460
5	0.380	0.440	0.420	0.440	0.400	0.480	0.480	0.400	0.460

Appendix 6: *Raw Data Collection for NiSO4 Solutions*

Tap Water			
Molarity	Seed Mass (± 0,01g)		
Time (Days)	Trial 1	Trial 2	Trial 3
0	0.190	0.190	0.190
1	0.380	0.390	0.490
2	0.400	0.400	0.480
3	0.530	0.410	0.500
4	0.530	0.470	0.520
5	0.590	0.530	0.550

Appendix 7*: Raw Data Collection for the Control Group*