TED ANKARA COLLEGE FOUNDATION SCHOOLS

Investigating the effect of increasing iron content in soil on the increase in biomass of *Zea mays* plants.

Extended Essay (Biology)

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Abstract

It is known that if there is not enough amount of a nutrient in the soil to meet the plants demand, that nutrient acts as a limiting agent and limits the development of the plant. Nutrients are divided into two; macro and micro elements. Macro elements are needed in greater amounts than micro elements. This experiment was aiming to show how the increasing iron in soil affected biomass of plant and to see, since iron is a micro element too, if it would make a significant difference. Iron is used in chlorophyll synthesis which is found in chloroplast, the organelle that contributes to increase in biomass of the plant, deficiency of iron causes chlorosis in leaves where they appear yellowish instead of green. There were 5 different groups which represent different iron contents in soil and 5 pots for every group, each pot containing two maize plants.

The experiment showed that the relation between iron amount and biomass is significant. The highest biomass was observed when maximum amount of iron was applied which is 40 ppm, the minimum biomass was observed when no iron was applied. The percentage difference between maximum and minimum biomass is 18%. Generally low standard errors make the obtained data very reliable and the results are consistent with other similar works done before. From the results of this experiment, it can easily be said that as the iron content in soil increases, biomass of the plants increase as well, the most probable cause of this being increased chloroplast number.

Introduction

When I started to think about my extended essay subject, the first thing I could think of was that I wanted to work with plants because I have always loved gardening. The first lesson I learned about the plants was that just watering them is not enough and you also have to fertilize them because soil loses minerals as the plants use them. I became curious and made a research on the internet; I learned every mineral deficiency affects the plant differently and has different symptoms.

As I learned, most of the time mineral deficiency can be diagnosed by examining the leaves[1], I started to wonder which other parts of the plant are harmed by this. Apparently, shoot and root growth was negatively affected[2] and the appearance of the plant was unhealthy. But all these are the results of different mineral deficiencies; I was mostly interested in iron deficiency because of a story I heard. The story says that there was an English woman who wanted to hang the laundry on a rope outside. She saw a tree with yellow leaves in her garden, thought the tree was about to die so she didn't hesitate putting a nail in the tree and tying one end of the rope to the nail. Later, she realized that the tree began getting better and she was surprised, went to the officials and expressed her shock, asked them to examine how was that possible. The examination showed the tree was suffering from iron deficiency before and the iron nail provided enough iron for the tree to recover. Today, more efficient methods are being used instead of iron nails like applying inorganic Fe salts to the soil[3].

I learned that in 1828 a principal called Liebig's Law of the Minimum was developed. This law states that if the soil has optimum amount of every element except one, the deficiency or absence of that element affects the growth and yield negatively. Plant growth and the yield are proportional to the amount of the missing element and that element becomes the limiting agent[4].

Elements are grouped as macro and micro elements by this law according to the needed amount of that element for healthy plant growth and development. Macro elements are needed in greater amounts, examples to macro elements can be nitrogen, calcium and magnesium. Micro elements are the ones needed in smaller amounts like iron and copper. There is a third group of elements which are accepted to be needed by certain plants, called functional nutrition elements[4].

Micro elements may be needed in smaller amounts when compared to macro elements but they are as important as macro elements for the healthy growth of the plant. Iron, the micro

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[1] Wallace, Thomas. "The diagnosis of mineral deficiencies in plants by visual symptoms. A colour atlas and guide." *The diagnosis of mineral deficiencies in plants by visual symptoms. A colour atlas and guide.* (1943).

[2] Cakmak, Ismail, Christine Hengeler, and Horst Marschner. "Partitioning of shoot and root dry matter and carbohydrates in bean plants suffering from phosphorus, potassium and magnesium deficiency." *Journal of Experimental Botany* 45.9 (1994): 1245-1250.

[3] Aydemir, Orhan, and Faruk Ince. *Bitki Besleme(Plant Nutrition)*. Diyarbakır: Dicle Universitesi Egitim Fakültesi Yayınları, 1988. Print.

[4] Karaman, Mehmet R. *Bitki Besleme(Plant Nutrition)*. Ankara: Gübretaş Rehber Kitaplar, 2012. Print.

element I want to work with is also very important for healthy plant growth. It is used as a catalyst in chlorophyll synthesis and used in many reactions because of it's ability to be both oxidized and reduced. It is also required for the creation of nodules[4].

Iron deficiency reveals itself by chlorosis, leaves having yellowish colour instead of green, due to failure and reduction in chlorophyll synthesis. The symptoms first appear on the young leaves. The chemical composition of the leaves damaged chlorosis is also different from a healthy leaf[3].

Dry mass is the mass of an organism after all the water it contains has vaporised. Since I know from my biology classes that chlorophyll is a pigment that is found in chloroplast, which provides increase in mass and iron deficiency may lead to insufficient chlorophyll synthesis, I thought it might affect the dry mass of the plants too. This paper will focus on answering the research question: Is the dry mass of the plant *Zea mays* affected by the amount of iron in its soil?

I chose the plant *Zea mays*, which is known as maize or corn, of the order Poales, family Poaceae and genus Zea for my experiment. The variety of *Zea mays* will be used is Samada-07 which is said to have little tolerance for iron deficiency by Variety Registration and Seed Certification Centre.

Hypothesis

It is known that maize needs to be supplied with enough of the minerals it needs just like any other plant for healthy plant growth. Iron, as a micro element, is needed in smaller amounts than macro elements but its deficiency causes serious problems the most significant one being chlorosis starting in young leaves[3]. As mentioned before, iron is used in carrying on many life functions because of its ability to be reduced and oxidized. It is also used in the process of chlorophyll synthesis[4]. If the plant can't synthesise chlorophyll, it can't carry on one of the most basic functions in order to survive. This negatively affects the growth of the plant and the yellow appearance of the leaves due to lack of green pigment chlorophyll, which is simply called chlorosis, will make the plant look unhealthy overall. If the plant can't carry on photosynthesis, its increase in dry mass will be negatively affected too.

Plants take iron with their roots but it is known that some other ions like magnesium, calcium and zinc may competitively decrease iron intake[3]. Or simply the soil may not be able to supply enough iron to the plant. There may be many reasons explaining why the plant suffers from iron deficiency but they all result in the same situation, an unhealthy plant with leaves affected by chlorosis. It has also been observed that iron deficiency decreased sugar, starch and protein quality of potatoes[5]. By using the information gathered, it can be hypothesised that a plant with insufficient iron supply will have a smaller dry mass than a plant with sufficient iron supply.

[5] Chatterjee, C., Rajeev Gopal, and B. K. Dube. "Impact of iron stress on biomass, yield, metabolism and quality of potato *(Solanum tuberosum* L.)." *Scientia horticulturae* 108.1 (2006): 1-6.

Method Development and Planning

The biggest problem encountered was to how to create controlled experiment conditions in a school lab. Pots required a large area and they had to be together so that each plant could get equal amount of sunlight, every condition except iron content of soil had to be the same for each pot. Schools labs couldn't provide a space large enough, direct sunlight, needed materials and safety of plants because other students use the laboratories and may disturb the experiment.

To provide the elements mentioned, I looked at the web page of the Ankara University Faculty of Agriculture where I found contact information of a professor, Mr.Taban, contacted him and asked if their laboratory was available. His answer was positive so the place to keep the plants was found; only problem left was the materials. The most important part of planning was how to control the iron content of soil. Faculty had a reserve of soil, taken from different areas of the country and of course with different content. Small samples of different soils were prepared for analysis in ICP/OES machine; by this analysis detailed content of soil can be learned. To prepare the soils, first of all they were dried in a dark area; dried soils were pressed on until the big particles were chipped off. After analysis, soil from Haymana district of Ankara seemed the best choice for the experiment. Its texture is clay loam, it contains 34.66% lime, which decreases the iron intake of the plant and 1.2 ppm iron, which professor said was low and combined with the amount of lime, iron deficiency could easily be observed. Calcareous soil has high pH, because of HCO_3^- concentration and elevated pH level decreases iron uptake of plants[6]. This is because as pH increases, critical redox potential of iron decreases, causing its solubility to decrease[7]. This property of the soil made it very suitable to observe iron deficiency easier.

To supply iron to groups other than control group, an iron solution had to be prepared. $Fe(So_4)_3.7H_2O$ is decided to be used for preparing the solution because unlike other iron compounds in the laboratory, its solubility is more satisfactory. Additional nitrogen, phosphorus and potassium were also given to ensure plants have enough of these minerals and they only suffer from iron deficiency. To make sure mineral content of soil wasn't affected by water, plants were watered with purified water instead of tap water. To prevent contact between soil and outer environment, including the pot, soil is placed into clear plastic bags.

Greenhouse of the faculty was chosen to keep the plants in because it was sunny during most of the day and plants could take direct sunlight. Plants were randomly placed on the table after they germinated to minimize the shading effect they have on each other with changing angle and direction of sunlight throughout the day. One major problem with greenhouse was the daily temperature change from night to noon was up to 7◦C as a thermometer that shows the highest and lowest temperatures showed. To keep the temperature stable a method was developed: keeping the glasses and the floor of the greenhouse wet helped decreasing the temperature for about 3◦C during day and when it started to get dark, I carried plants to inside sections of the greenhouse which was darker and therefore about 2◦C colder than outside sections. This method helped ensuring that plants didn't go through a dramatic temperature change and take the maximum amount of sunlight.

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[6] Barker, Allen V., and David J. Pilbeam, eds. *Handbook of plant nutrition*. CRC press, 2010.

[7] Kacar, Burhan and Katkat, A. Vahap. *Bitki Besleme(Plant Nutrition)*. Vipaş Yayınları, 1998. Print.

Plants were watered twice a day, at 11:00 and 17:00. 11:00 was chosen because it was just before the noon, when temperature started to increase and 17:00 was chosen because it was the time when rise of the temperature stopped and most of the water in soil was either vaporized or used by plants. 100 ml. of water was used in the first week after germination as there were five plants in each pot, after removal of three, amount of water was decreased to 50 ml. for two weeks and beginning from third week until the end of the experiment, amount was increased to 100 ml. again since plants needed more water as they grew.

When first planting the seeds, five seeds were planted into each pot as an insurance in case some of them didn't germinate but this was done without the knowledge of seeds being guaranteed 100% germination rate, after this was found out and all of the seeds germinated, three plants from each pot was removed at the end of first week because five plants were too much for the chosen size of pots. When removing plants, height of plants, width of body and size of leaves are used to determine the two plants closer to the average of the plants in that pot. When removing plants it was observed that seeds were still whole and connected to plants, roots were not fully formed which showed that plants were still using the seed as the source for minerals and therefore they didn't affect the mineral content of soil.

To measure dry mass, plants were cut after two months and left in an incubator for a week so that they would lose all the water in their tissues and only what creates dry mass is left. Plants were cut two centimeter above soil surface so that the whole body of plants can be weighed. I learned about the dry-weight method in Environmental Systems and Societies lesson while we were studying ecosystems.

Picture 1: Arrangement of the seeds when planting.

Picture 2: Arrangement of pots in greenhouse.

Materials and Apparatus

1 graduated cylinder (100 cm^3)

25 number 6 plastic pots (180 x 165 cm^3)

125 *Zea mays* Samada – 07 seeds

purified water

25 tags

25 clear plastic bags (medium size)

21.73 g urea

17.55 g KH_2PO_4

4.71 g iron sulphate

50 kg soil from Haymana district of Ankara

tweezers

glass rods

string

scale

dispenser

25 paper bags

Method

-A clear plastic bag was placed inside every pot and then the pot was placed on the scale. 2 kg of soil was added to every pot. The soil pH was 7.83.

-After pots are filled, fertilizers were prepared. For nitrogen, 21.73 g of urea was dissolved in 500 ml of purified water and 100 mg/kg nitrogen was given to soil by the help of a dispenser.

-Then, 17.55 g of KH_2PO_4 was dissolved in 500 ml of purified water; soil was given 40 mg/kg phosphorus and 50 mg/kg potassium by using a dispenser.

-Finally, 4.71 g of iron sulphate was dissolved in 1 l of purified water. This solution was not given to five pots of control group which later will be referred as Fe_0. To five pots of second group, 5 mg/kg of iron solution was given and this group will be referred as Fe_5, to five pots of the third group, 10 mg/kg of iron solution was given and this group will be referred as $Fe_{10,}$ to five pots of the fourth group which will be referred as Fe_{20}, 20 mg/kg of iron solution was given and to the remaining five pots, 40 mg/kg of iron solution was given and this group will be referred as Fe_{40}.

-Each pot was tagged with group name.

-After fertilization, ends of the bags were tied together so they stay closed and evaporation was prevented and soil can absorb solutions.

-Next day, after soil absorbed the solutions, each soil was mixed inside its own plastic bag so that the solution could spread to every part of the soil equally.

-When mixing was done, five Samada – 07 seeds per pot were planted about a centimeter under surface.

-180 ml of purified water was used per pot for the first watering. Then, pots were covered with a newspaper and left for three days for germination.

After three days, it was observed that all seeds had germinated.

-The plants were watered with 100 ml of water per pot and a week after germination; three plants from each pot were removed with tweezers. Tweezers were also used to remove unwanted plants growing inside soil. After removal of plants, the amount of water was decreased to 50 ml and in the fourth week increased back to 100 ml. The temperature of soil was 25◦C before watering and 23.5◦C after watering.

-Then the pots were randomly placed on the table to minimize the effect of their position on their development.

-As plants grew, amount of water given was increased from 50 to 100 ml per pot. The glasses of the greenhouse and the floor were wetted every half an hour to maintain a constant temperature inside the greenhouse. Around 6 pm, pots were carried one by one to the darker section of the greenhouse which was also colder so that there wouldn't be a big temperature difference in the night, outer section of the greenhouse was tried to be kept at the same temperature of the inner section during the day, which was 27◦C. Same process was continued for two months.

-After two months, plants were cut about 1 centimeter above soil surface, rinsed with purified water and tied into a knot shape then placed into paper bags. Plants of the same pot were put in the same bags.

-Bags were marked with group name of the plant and then bags were placed in an incubator which in this case, served as an oven.

-Plants were left in the incubator for ten days, during this time all water in plant's tissues are lost due to high temperature. Mass of the plant after water vaporizes is called as dry mass, which was what we intended to compare in this experiment.

-Dry mass was measured by using a scale.

Picture 3: Cleaning the leaves and the body in purified water before placing in the incubator.

Picture 4: Leaves and body of a plant after it is removed from the incubator.

Data Collection & Processing

Raw Data Table 1. Raw data table showing biomasses of plants, iron content of the soil as the independent variable and constant variables of soil pH, temperature of the environment, amount of applied nitrogen, urea, potassium and phosphorus.

	Biomass (g/pot) (±0.05)					pH of soil (±0.005)	Temperature of the environment (◦C) (±0.05)	Amount of nitrogen applied (mg) (±0.5)	Amount of urea applied (g) (±0.05)	Amount of potassium applied (mg) (±0.5)	Amount of phosphorus applied (mg) (±0.5)
Amount of iron applied to the soil (ppm)/trial	0 (±0.05)	5 (±0.05)	10 (±0.05)	20 (±0.05)	40 (±0.05)						
1	4.98	5.21	5.69	5.10	5.29	7.83	27.40	200.0	21.73	100.0	80.0
2	5.22	4.54	5.24	5.17	5.48	7.83	27.40	200.0	21.73	100.0	80.0
3	4.65	5.22	5.15	5.30	6.21	7.83	27.4 0	200.0	21.73	100.0	80.0
4	4.47	5.04	4.78	6.01	6.40	7.83	27.40	200.0	21.73	100.0	80.0
5	5.11	5.22	5.37	4.84	5.46	7.83	27.4 0	200.0	21.73	100.0	80.0

Table 2. A summary of the results presented in Table 1., showing the mean of biomasses of the plants of each group and percentage increase of each group when compared to control group.

Amount of iron applied to soil (ppm)	0 (±0.05)	5 (±0.05)	10 (±0.05)	20 (±0.05)	40 (±0.05)
Mean biomass	4.89	5.05	5.25	5.28	5.77
% Increase in biomass	0	3.27	7.36	7.98	18.00

Data Analysis

Table 4. Table showing the statistical calculations of the experimental data.

	Fe_0	Fe_5	Fe_{10}	Fe_{20}	Fe_{40}
Mean	4.89	5.05	5.25	5.28	5.77
Variance	0,09983	0,08588	0,10973	0,19283	0,25027
Standard Deviation	0,31596	0,29305	0,33126	0,43912	0,50027
Standard Error	0,1413	0,13106	0,14814	0,19638	0,22373
T-value	2,7765	2,7765	2,7765	2,7765	2,7765
95% Confidence Interval	0,39232	0,36388	0,41132	0,54526	0,62118

Table 5. Results of ANOVA test.

Summary

Groups	*Count*	*Sum*	*Average*	*Variance*
Fe 0	5	24,43	4,886	0,09983
Fe 5	5	25,23	5,046	0,08588
Fe 10	5	26,23	5,246	0,10973
Fe 20	5	26,42	5,284	0,19283
Fe 40	5	28,84	5,768	0,25027

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2,21764	4	0,55441	3,753418908	0,019552844	2,866081402
Within Groups	2,95416	20	0,147708			
Total	5,1718	24				

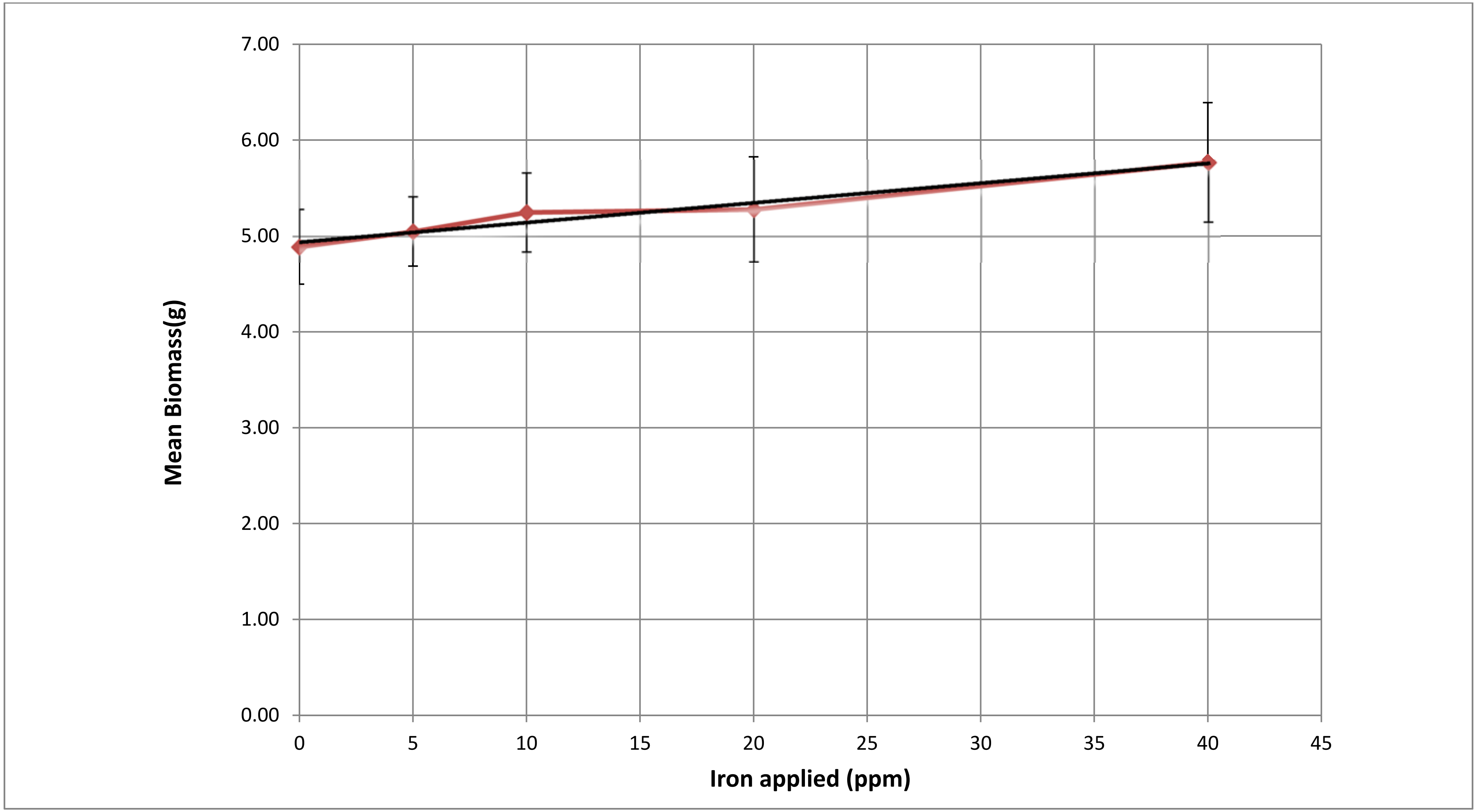
H_0: There is no difference in mean biomass of groups of plants which grow in soil that has different iron concentration.

H_1: There is a difference in mean biomass of groups of plants which grow in soil that has different iron concentration.

$p=0.019552844$

$p<0.05$

H_1 is proved.



Graph 1. Graph showing relation between mean biomass of *Zea mays* and amount of iron applied to the soil, best fit line and error bars. Amount of other minerals and the room conditions are kept constant.

Conclusion

In this experiment, the question “Is dry mass of the plant *Zea mays* affected by the amount of iron in its soil?” was tried to be answered. It was hypothesized that relation between biomass and iron content would be directly proportional because iron is used in chlorophyll synthesis, which is used in photosynthesis, therefore has a very important role for the survival and growth of plants.

There were five groups of different iron content in soil, five pots of each group and two plants in each pot. A soil with low iron content was found to be the best choice for the experiment. Different iron levels were maintained by applying different amounts of additional iron and basic fertilization was applied which included nitrogen, potassium and phosphorus elements, to make sure the plants didn’t suffer from any other deficiency .The duration of the experiment was two months, during this time plants were kept in a stable environment. Results were evaluated as biomass per pot instead of biomass of each plant individually because it is very possible for one of the two plants in a pot to take up more minerals from the soil based on its position, root development and many other factors. As the iron content in soil increased, it was observed that biomass of the plant was increased as well.

The experiment results are, mean biomass for Fe_0, Fe_5, Fe_{10}, Fe_{20} and Fe_{40} groups respectively are, 4.89, 5.05, 5.25, 5.28 and 5.77 grams. These results support the hypothesis that mean biomass of *Zea mays* plants is affected by iron concentration of soil. Actually, they prove the hypothesis by showing the biomass of plants are increased when they are planted in a more iron rich soil and there is an 18% difference between the control group and the most iron rich group. ANOVA test shows that p value is 0.019, lower than 0.05, which means that the possibility of getting these results just by chance is 19 in a thousand trials, which is low enough to say that the results show the actual relation between iron content and biomass.

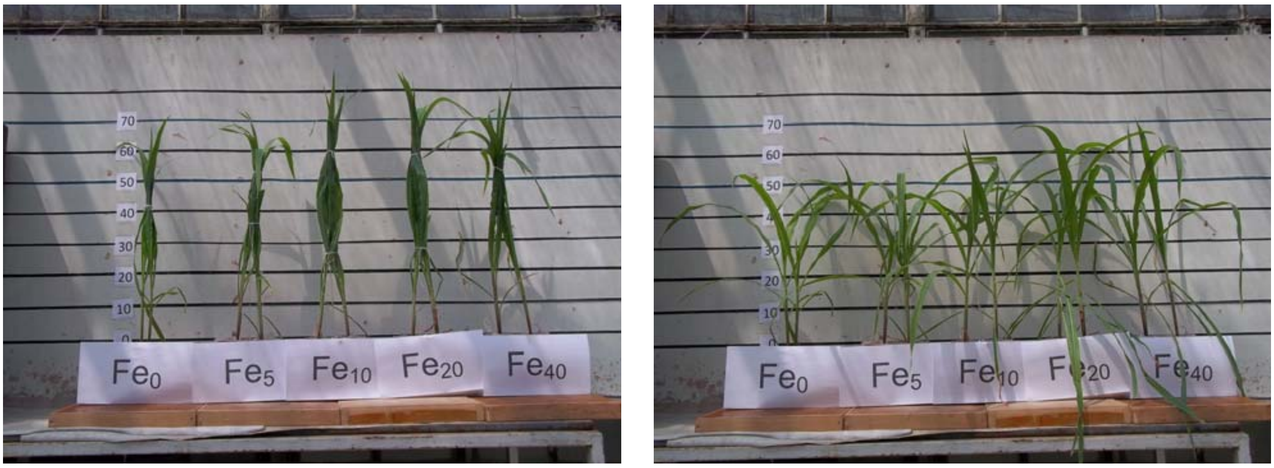
Results of the experiment are presented in a mean biomass vs. amount of iron applied line graph. The error bars for Fe_{40} group are bigger than other groups’ because of high standard error. The points on the graph represent the results obtained by analyzing experiment results. The best fit line passes through three of these points and passes very close to the other two, which shows the accuracy of obtained results is high. The graph proves the hypothesis right by showing an increase in biomass as the amount of iron applied increases.

In an investigation[8] by Süleyman Taban and Mehmet Alpaslan from the Ankara University Faculty of Agriculture to determine which iron compounds are more effective on the development of maize plant, they applied iron by using different compounds and different amounts of each compound; one of the dependent variables they measured was the biomass. Their results show that as iron content of soil increased, biomass of plants increased as well but after a point, too much iron started to create a toxic effect on plants. My experiment didn’t show this toxic effect of excess iron, there may be many reasons where it is most likely caused by the different amounts of iron applied, if my experiment had another group that I applied even more iron, toxicity might have been observed

[8] Taban, Süleyman, and Aplaslan, Mehmet. “Değişik Form ve Miktarlarda Uygulanan Demirin Mısır Bitkisinin Gelişmesi ve Bazı Mineral Madde Kapsamları Üzerine Etkileri. (Effect of Iron Applied In Diferent Forms and Amounts on Development of Maize Plant and Some Mineral Scopes)” 1991. Print.

in this experiment as well. Taban and Alpaslan cited Singh and Sinha's work[9] as their investigation shows similar results, they also observed that increasing amount of iron in soil caused an increase in biomass. These similar results from other investigations show the results of the experiment are accurate.

To have a better understanding of the effect of the amount of iron applied on biomass of the plants and the accuracy of the experiment, results were statistically examined. One thing about this examination is noticeable that standard deviation and standard error for Fe_{40} which are respectively 0.50027 and 0.22374 are relatively higher than the other groups. I placed plants randomly to spread them and prevent the same group plants to be close to each other so that if their position effected their development, this external intervention would not affect the whole group and lead to wrong results. Some plants took more sunlight than the others, this caused their soil to dry faster by increasing the evaporation of water and this might have negatively affected the plants, the fact that most of the Fe_{40} group plants were in this area can explain the noticeably high standard error, the results in this group varies more than the others.



Picture 5 and 6: One plant from each group right before they were cut.

Picture 7: The plants before they were cut and me.

[9] Singh, R., Sinha M.K. "Reactions of Iron Chelates on Calcerous Soil and Their Relative Efficiency in Iron Nutrition of Corn." Plant and Soil, 46, 1977. Print.

Evaluation

Even though one of the main purposes of using the greenhouses of the university was to ensure the safety of the plants and to provide the most stabilised conditions, the second goal could not be fully achieved. The Faculty had air-conditioned green houses which could have provided the most stable and controlled environment but during the time the experiment was carried on, they were on full capacity so they couldn't be used. In previous sections, it was mentioned that the glasses and the floor of the greenhouse was kept wet to minimize the temperature change between day and night and plants were carried to the inner section of the greenhouse which was colder than the outer section the plants were kept during day time. I tried to keep the temperature during the day as close as I can to the night temperature, despite all the effort there was still temperature difference but the difference wasn't big, the most stable conditions could be created if it was possible to use the air-conditioned greenhouses.

After the second week, some small spots and holes were observed on the leaves of the most plants. I consulted to the professor which provided me the materials for this unexpected observation; I learned that these symptoms were caused by a parasite. The seeds were containing pesticide to keep the seed safe when storing it but this pesticide didn't have any protection on the plant itself after germination. The parasite infection didn't increase so I didn't consider it necessary to apply pesticide because with all the ingredients in contains, it could have affected the controlled conditions like mineral ingredient of the soil. The parasite infection wasn't present only in a particular group and it wasn't advanced so although it might have lead to decrease in biomass in infected plants, it can be considered negligible.

The biggest problem in this experiment was caused by the unwanted plants growing in the pots. These plants were already present in the soil before the experiment and they germinated when they found a suitable environment. These plants could have a great intake of iron and cause a stronger deficiency in plants than desired. I removed these unwanted plants by using tweezers when they germinated with the maize plants but the best thing to do would be placing the soil in pots and water it, cause these plants to germinate, remove them using tweezers and then add minerals and seeds to the soil so that the unwanted plants wouldn't be able to benefit from the nutrients applied for the maize plants.

The maize plant has a very important place in economy. It is one of the most used plants in human diet besides wheat. Almost every food product on the market shelves, there are starch, oil or syrup which comes from maize. It has such a vital importance in food industry that lots of research had been done to make it resistant to insects and diseases. There is a big demand for maize and farmers need to get maximum yield from their crops to meet with this demand. Healthier, more developed plants mean more yield. Annual soil analyses should be done to see if the soil can support development of the plant or if the low mineral content would be a limiting agent in plant development.

Although this experiment proved that iron has an important role on the increase in biomass, there are many macro elements like nitrogen, magnesium and micro elements like zinc and chlorine which can affect biomass too. Iron on its own can't be taken responsible for the increase in biomass, other elements can be tested also to find out which has the greatest impact on the increase in biomass.

Appendix

<u>Soil content analysis</u>

The soil used for the experiment was taken from Haymana district of Ankara in fall season.

Soil samples were placed on a cover, dried in an area without sunlight.

Dried samples were put into plastic bags and pressed on until the big particles separated.

Soil was passed through 2 millimeter sieve and put into glass containers with closed lids to prevent contamination.

A part of the sample was analysed in ICP-OES device to learn iron content.

A part of the sample was analysed by using Scheibler Calcimeter to learn lime content.

<u>Calculating dry mass</u>

The plants were cut one centimeter above the surface level and rinsed wit purified water.

Rinsed plants were tied into a knot and placed into paper bags and each bag was tagged according to the iron group the plant was from.

The plants inside bags were placed into an incubator which has high temperature inside; therefore all water is vaporized from the plant tissue, leaving behind what is called the dry weight biomass.

The plants were taken out of the incubator after ten days and each plant was weighed, which gives the biomass.

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