Investigating the effect of heavy metal water on the photosynthesis efficiency in tomato plants

word count: 3125

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INTRODUCTION

By the aid of technological and scientific achievements, humanity has to a great extent, achieved in establishing and operating nature. They have plundered the natural resources, hence made those reserves available for their use. The averages of industrialization and urbanization have made life more comfortable without any doubt. In the means of manipulating and controlling nature, humanity has modified his biotic and abiotic environment according to their needs; while altering their surroundings, they have also dismissed some meaningful postulates that are governing the ecosystems. [1]

Water is essential for all living creatures. Nearly everything consists of water directly or indirectly. However, this very cherished water is getting dirtier day by day mostly because of humans. The damage given to the environment creates a kind of undesirable consequences to humans, who are also easily ignoring living beings' right to live.

Our planet's water supplies are limited. Erratic stock and contamination moreover limit the availability of water for various applications by individuals. People tend to gather the water from the water cycle for their viral and industrial obligations, but they then deliver the water back to the cycle once they have been used for their benefit. Throughout this process, the elements that got combined with the water adjust water's physical, chemical or/and biological characteristics and worsen the condition of water sources.

Water pollution occurs when a pollutant is discharged into water bodies without removing hazardous compounds by adequate treatment. Residential and industrial wastewater is most likely to be the antecedent, and about 70% of the reason behind of current water pollution is domestic wastewater. An increase in nutrient loads like an increase in fertilizer use might lead to eutrophication. Organic wastes, like city sewage, cause excessive oxygen burden on the receiving water system, leading to the result of depletion of oxygen which can have serious effects on the ecosystem. Industry releases wastewater including resin pellets, organic toxins, heavy metals, petroleum, nutrients, solids etc. These emissions are accompanied by the thermal motion, but also reduce the available oxygen. The effluent with the silt resulting from many actions including the construction site, forest logging, agriculture, limits photosynthesis, broadly covers the lake bottom and the bottom of the river, replaces the damaged ecosystem instead of the light shining through the water column, it inhibits penetration. [2]

Heavy metals are one type of pollutants in the water. They are spontaneously forming elements with a high atomic mass and density of nearly 5 times greater than water. [3] Noted origins of heavy metals in nature include geogenic, agricultural, industrial, pharmaceutical, domestic effluents, and atmospheric roots. [4] Due to their great level of toxicity; arsenic, cadmium, chromium, lead, and mercury are amongst the superiority metals that are of public health importance. They have been categorised as human carcinogens by the U.S. Environmental Protection Agency, and by the International Agency for Research on Cancer. [5] Communities are opened to heavy metals principally by water consumption, but several heavy metals can bioaccumulate in the human body and may provoke cancer and other dangerous diseases. Numerous developing nations are also met with the challenge of lessening human display to heavy metals, principally because of their insufficient budgetary abilities to utilise advanced technologies for heavy metal elimination.

“Heavy metals” is actually a joint phrase for metals with high atomic mass. Transition metals especially, that are also known to be poisonous and unqualified to be concocted by bodies, like mercury, lead, and cadmium. That idiom is usually accepted for metals and semimetals (metalloids) as a group name. [6] However, in everyday usage it suggests something different, it’s indicating a metal that is capable of causing health quandaries or environmental contamination.

Kızılırmak River, also known as the Halys River, is Turkey's longest river (1,355km) with approximately 5 million people living in the basin area. While some of the residential, agricultural and industrial water is being supplied by Kızılırmak, the resultant wastewater, of various human activities, is also flowing back into the river without being purified. This situation is causing the river water swiftly contaminated.

Recent studies showed that chemical, biological and radioactive materials are present in the water of Kızılırmak. Among them, various metals are harmful to the human body (such as lead, manganese, chromium, copper, and mercury) are caused by the waste of machinery and other industrial facilities. On the other hand, most of the residents in the basin area are drinking this water. In addition to this, many plants have been using this water to continue their metabolic activities and producing the basic need of other living things, oxygen.

Photosynthetic pigments transform light energy into chemical energy in photosynthetic living organisms. These pigments include; Chlorophyll-a, Chlorophyll-b, Xanthophyll, Carotene. [7]

Chlorophyll-a is the primary photosynthetic pigment present in plants and algae. It is the most widely distributed type of chlorophylls. It receives light mainly in the blue and red areas from the spectrum. Chlorophyll-b, Xanthophyll and Carotene are accessory photosynthetic pigments that are also present in plants and green algae. They receive light from other areas spectrum and transfer the light energy to Chlorophyll-a.

In this experiment, I’m planning to ascertain the alteration in the chlorophyll contents and also their photosynthesis efficiencies, varying with the type of water (tap water, and river water) and also find out how dangerous is Kızılırmak water to the living bodies around. I chose the plant tomato because recent studies have also done with this plant, as shown in Appendix 1.

So this investigation is aiming to address the research question: How do the Kızılırmak water and tap water from home affect the photosynthesis efficiency ratio of a tomato plant along wıth chlorophyll-a and chlorophyll-b concentrations?

HYPOTHESIS

Water contamination has a broad assortment of effects on plant metabolism and on the environment overall. Pollution in water not only affects negatively the plant growth but also leads plants to consume hazardous chemicals through the water and transfer the toxic substances to animals, by being eaten, that they rely on them for survival. Pollution can also have an influence on photosynthetic capacity and respiration rate of leaves of the plants. Photosynthesis is a necessary process which plants use the energy from light, received from the sun, to produce glucose, by transforming carbon dioxide taken into carbohydrates. However, when the water is poisoned, it's potential of dissolving gases like carbon dioxide is effected in a negative way. Since plants that grow in water or in hydrophytes earth depend on the photosynthetic process to keep continuance, an intervention to the photosynthetic process can eliminate them. In short, the water of the Kızılırmak River is most likely to cause chlorophyll to fall in value compared to the tap water according to previous researches (Appendix 1). It's also possible that the increasing density of pollutants may precipitate visible lesions such as a lack of advancement and germination difficulty. [8] Addition to this, the amount of tomato produced might be less or nearly none by the experimental group.

METHOD DEVELOPMENT

	Name of Variable	Method of management and/or measurement
Independent variable	➔ Type of water (tap and river)	➔ Graduated bowl/L ➔ Used for watering the plants
Dependent variable	➔ Chlorophyll concentration of the leaves of the tomato plants	➔ Calculated by the formulas of Lichtentaler and Wellburn by using lambda values
Controlled variable	➔ Type of plant ➔ Light received ➔ Pot volume ➔ Place where the pots are kept ➔ Volume of soil ➔ The volume of irrigation water given ➔ Type of soil ➔ The material of the pot	➔ Tomato plants used which bought from the same market ➔ The pots placed next to each other where they can get an equal amount of sunlight ➔ Dimensions of the flowerpots are 1x0.5x0.5m ➔ The pots will be located in the terrace of the house ➔ The soil used is 200 dm^3 ➔ The pots are identical ➔ In the beginning, 2L water will be given to saplings and increase it to 5-7L after 3 weeks. Irrigation will take place twice a week ➔ The soil used has the same brand and brought from the same marketplace ➔ The pots are made from the material wood
Uncontrolled variable	➔ Initial heights of the saplings	➔ Different initial lengths may cause small errors since they may cause differences in the rate of development of leaves

Table 1. List of independent, dependent, controlled and uncontrolled variables

The biggest problem is to transport river water from the Kızılırmak. For making river water available, I planned to use water canisters. In this way, it will become easier to water the plants. I will fill the canisters with the river water and store them for later, to water the seedlings.

I will place the saplings in an area where they could receive equal amounts of sunlight. The fact that their position is next to each other will help me reduce the error due to controlled elements. In addition, to reduce the error, I will try to plant the seedlings not too close to each other. I hope the size of the flowerpot I have (1x0.5x0.5m) will be enough for them to grow without inhibiting each other.

At first, I was not sure how to compare the change in chlorophyll levels, I was thinking of comparing the shades of green they have, but I was unable to calculate the shade of green. With some research, I found out that there are formulas for calculating chlorophyll-a, chlorophyll-b and total chlorophyll levels through lambda values. In addition to this, the formulas of Lichtentaler and Wellburn (1985) [9] allows calculating even the photosynthesis efficiency ratio.

To extract the chlorophyll content of the plant and calculate the lambda value, I need advanced equipment. For this, I got permission from a hospital laboratory to use their laboratory equipment (Appendix 2).

MATERIALS

Name of the Material	Size/Amount
Tomato seedlings	34
Gardening gloves	
Soil	200 dm^3
Flowerpots	1m x 0.5 x 0.5m / 2
Tap (Home) Water	100L
Kızılırmak Water	100L
Scissors	1
Weigher	1
Ethyl Alcohol (acetone can also be used)	4000ml
Graduated cylinder(s)	1
Crucible(s)	1
Test tubes	20
Rubber gloves	
Centrifuge CL-24	
Spectrometer	

Table 2. List of materials

PROCEDURE

1. Plant the saplings in each of the pots (1x0.5m) in equal number (17 to 17)
2. Keep watering the saplings 2 days a week for 2 months until the plants start to grow tomatoes
    a. Start giving 2L water for saplings and continue like this until they grow more leaves
    b. Increase the water volume to 5-7L after 3 weeks
3. Harvest the fresh leaf samples from each of the tomato plants, from middle parts of the plant
4. Cut the leaf sample to make them weight 100mg each
5. Crush 100 mg leaf in 20 ml of 80% ethyl alcohol
6. Centrifuge the extract for 10 min at 1000 rpm (revolutions per unit)
7. Place it the spectrophotometer
8. Record the absorbance of the supernatant at 645 and 663 nm
9. Repeat the procedures 4-8 for every leaf sample
10. Use the formulas of Lichtentaler and Wellburn (Appendix 3) to calculate chlorophyll-a, chlorophyll-b and total chlorophyll levels
11. Write down the data gained

RESULTS

		Absorbance of supernatant (λ±0.001)	
		663 nm	645 nm
Kızılırmak Water	1	0.110	0.062
	2	0.132	0.078
	3	0.121	0.080
	4	0.094	0.052
	5	0.080	0.056
	6	0.105	0.078
	7	0.095	0.065
	8	0.124	0.081
	9	0.150	0.071
	10	0.119	0.075
Tap Water	1	0.583	0.253
	2	0.641	0.275
	3	0.669	0.288
	4	0.552	0.240
	5	0.668	0.278
	6	0.596	0.310
	7	0.612	0.284
	8	0.597	0.251
	9	0.610	0.263
	10	0.650	0.290

Table 3. The absorbance of the supernatant at 645 and 663 nm

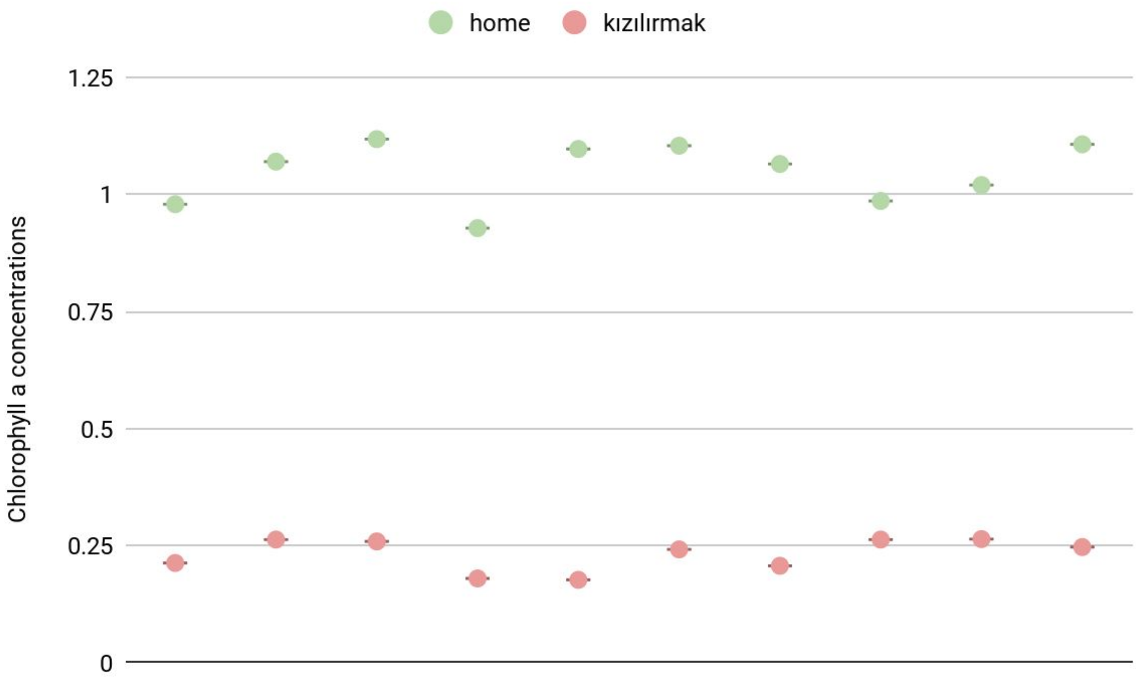
DATA PROCESSING

The formulas of Lichtentaler and Wellburn (Appendix 3) is used:

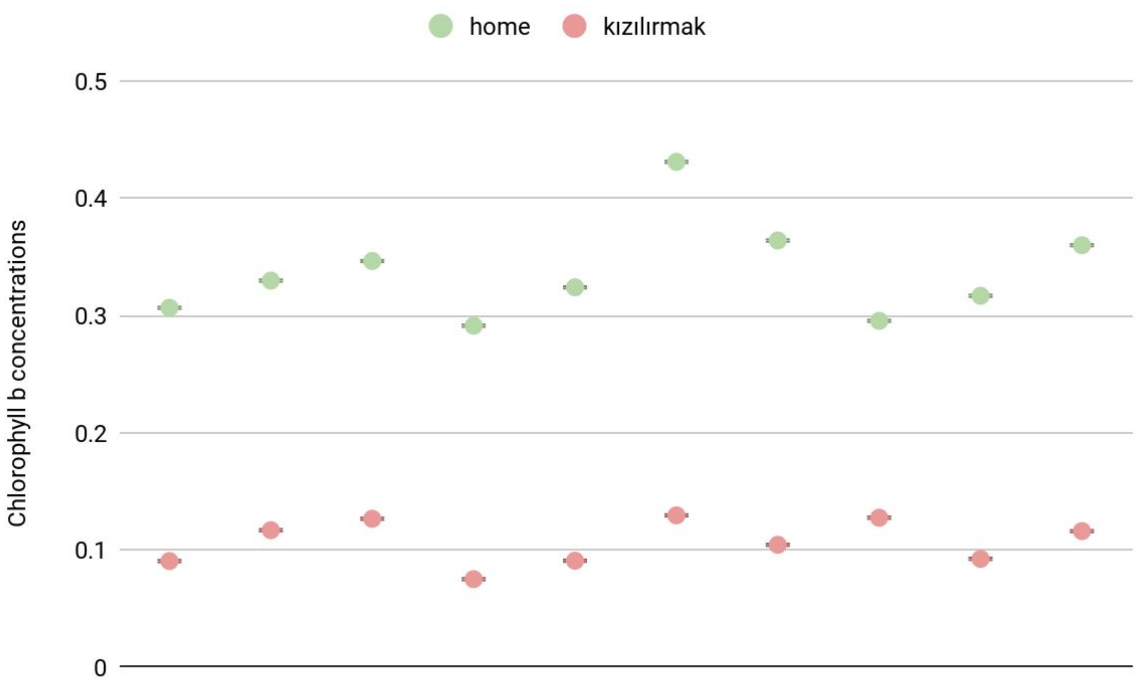
Chlorophyll-a	= 12.7 (A663) – 2.69 (A645) x VW = 12.7 (0.110) – 2.69 (0.062) x 10 = 0.123
Chlorophyll-b	= 22.9 (A645) – 4.86 (A663) x VW = 22.9 (0.062) – 4.86 (0.110) x 10 = 0.091
Total chlorophyll concentration	= [20.2 (A645) – 8.02 (A663) x VW] / 1000 = [20.2 (0.062) – 8.02 (0.110) x 10] / 1000 = 0.213
Photosynthesis efficiency ratio	= [12.7 (A663) – 2.69 (A645) x VW] / [22.9 (A645) – 4.86 (A663) x VW] = [12.7 (0.110) – 2.69 (0.062) x VW] / [22.9 (0.062) – 4.86 (0.110) x 10] = 1.359

		Chlorophyll-a $\left(\frac{mg\ chl\ a}{gr\ tissue}\right)$ ($\pm$0.001)	Chlorophyll-b $\left(\frac{mg\ chl\ b}{gr\ tissue}\right)$ ($\pm$0.001)	Total Chlorophyll concentration (mg/g1) ($\pm$0.001)	Photosynthesis efficiency ratio (chl a/ chl b)
Kızılırmak Water	1	0.123	0.091	0.213	1.359
	2	0.147	0.117	0.263	1.255
	3	0.132	0.127	0.259	1.044
	4	0.105	0.075	0.180	1.404
	5	0.087	0.091	0.177	0.953
	6	0.112	0.129	0.242	0.868
	7	0.103	0.104	0.207	0.988
	8	0.136	0.127	0.263	1.065
	9	0.171	0.092	0.264	1.855
	10	0.131	0.116	0.247	1.128
Tap Water	1	0.672	0.307	0.979	2.193
	2	0.740	0.330	1.070	2.244
	3	0.772	0.346	1.118	2.229
	4	0.636	0.291	0.928	2.185
	5	0.774	0.324	1.097	2.388
	6	0.674	0.431	1.104	1.563
	7	0.701	0.364	1.065	1.926
	8	0.691	0.295	0.986	2.338
	9	0.704	0.317	1.020	2.222
	10	0.747	0.360	1.107	2.077

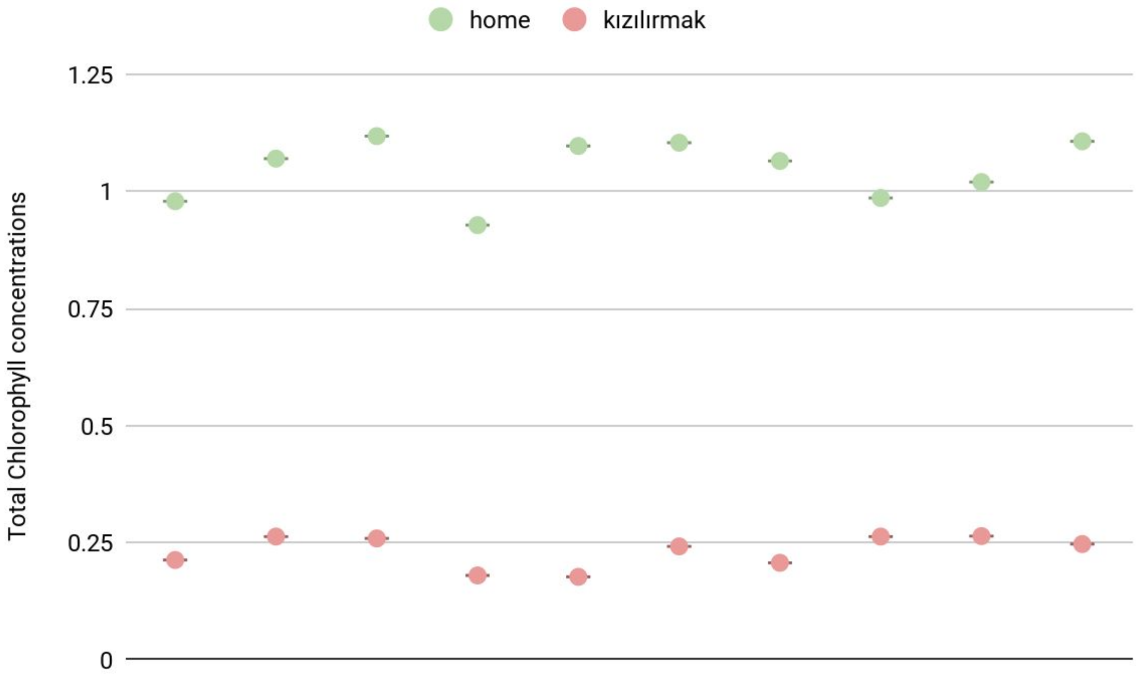
Table 4. Chlorophyll-a, chlorophyll-b, total chlorophyll concentration and photosynthesis efficiency ratio obtained by formulas of Lichtentaler and Wellburn



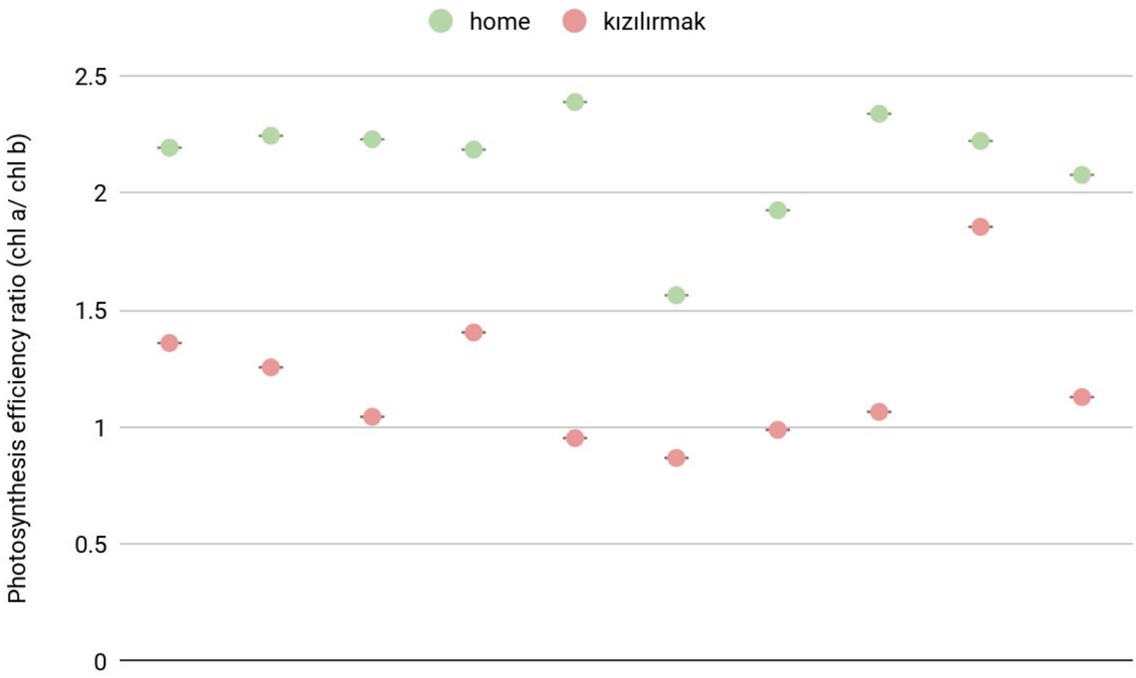
Graph 1. Graph of distribution of chlorophyll-a values



Graph 2. Graph of distribution of chlorophyll-b values



Graph 3. Graph of distribution of total chlorophyll values



Graph 4. Graph of distribution of photosynthesis efficiency ratios

Mean:

Formula: $\frac{\textit{data values}}{\textit{number of trials}}$

$$\frac{0.123 + 0.147 + 0.132 + 0.105 + 0.087 + 0.112 + 0.103 + 0.136 + 0.171 + 0.131}{10}$$
$$= 0.125$$

Standard Deviation:

Formula: $\sqrt{\frac{\Sigma|x-\mu|^2}{N}}$

(μ =mean / N=number of data / x=individual values)

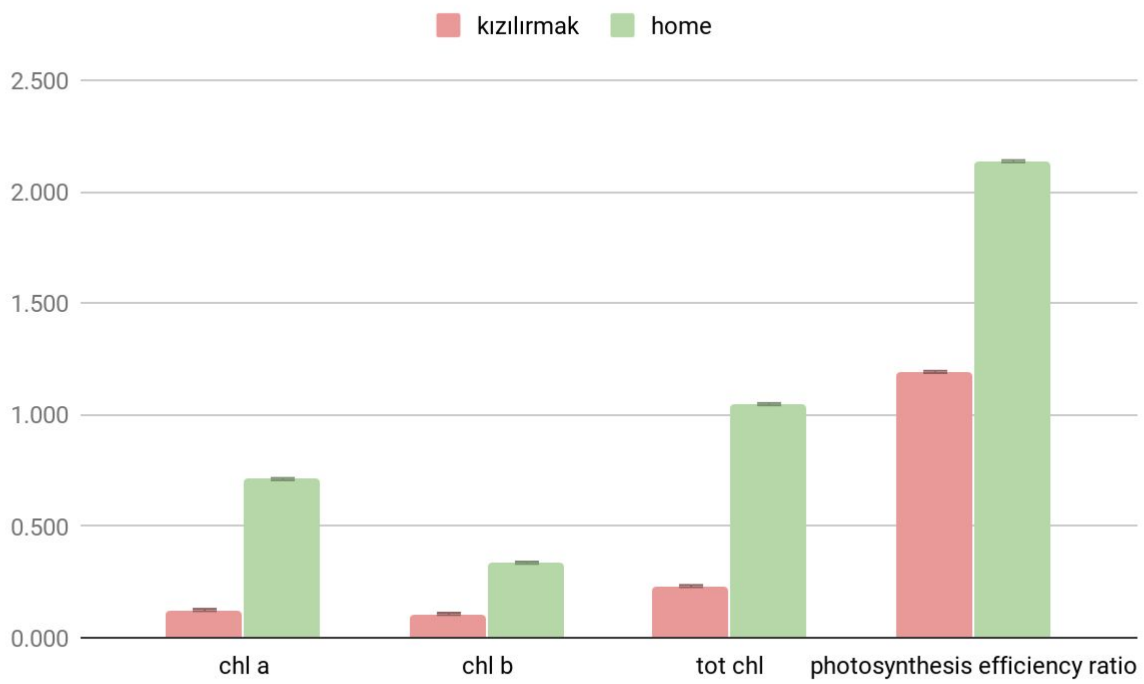
$$\sqrt{\frac{\Sigma|x-0.125|^2}{10}} = 0.024$$

		Chlorophyll-a	Chlorophyll-b	Total Chlorophyll Concentration	Photosynthesis Efficiency Ratio
Kızılırmak Water	Mean	0.125	0.107	0.232	1.192
	Standard Deviation	0.024	0.019	0.034	0.291
Tap Water	Mean	0.711	0.336	1.047	2.137
	Standard Deviation	0.046	0.042	0.066	0.239

Table 5. Table of mean values and standard deviations of chlorophyll-a, chlorophyll-b, total chlorophyll concentration and photosynthesis efficiency ratio

The mean is calculated to show the differences in chlorophyll-a, chlorophyll-b, total chlorophyll concentration and photosynthesis efficiency ratio easier, in this way I'm planning to show the results altogether instead of drawing individual graphs for every data which may also create confusion.

The standard deviation calculated is small, and means that the data gathered are not varying too much and accurate. The standard deviation calculated is used to form the error bars at the final graph drawn.



Graph 5. Graph of mean values of chlorophyll-a, chlorophyll-b, total chlorophyll concentration and photosynthesis efficiency ratio

Error bars represent standard deviation. As the error bars of the two groups do not overlap in an independent variable, it can be said that there is a difference between the mean values obtained upon the given independent variables

DATA ANALYSIS

H_0: There is not a statistically significant difference between the mean photosynthetic efficiency ratios of tomato plants irrigated with Kızılırmak and home water.

H_1: There is a statistically significant difference between the mean photosynthetic efficiency ratios of tomato plants irrigated with Kızılırmak and home water.

t-Test: Paired Two Sample for Means		
	kızılırmak	home
Mean	1.191945684	2.136528049
Variance	0.08471560717	0.05710431949
Observations	10	10
Pearson Correlation	0.3463235067	
Hypothesized Mean Difference	0	
df	9	
t Stat	-9.761113486	
P(T<=t) one-tail	2.18717E-06	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	4.37434E-06	
t Critical two-tail	2.262157163	

Table 6. Table for t-Test: Paired Two Sample for Means

Results for t-Test: paired two samples for means. As the p-one tail value which is 2.18717 x 10^{-6} is smaller than the α value which is 0.05, H_0 is rejected and H_1 is accepted. So There is a statistically significant difference between the mean photosynthetic efficiency ratios of tomato plants irrigated with Kızılırmak and home water.

CONCLUSION

The standard deviation is low so I decided it wouldn't be showing a great difference if I draw the graphs for all of the data individually. According to mean values and as seen in graph 5, the Kızılırmak water which includes heavy metals, decrease significantly all chlorophyll-a, chlorophyll-b and total chlorophyll levels and so that the photosynthesis efficiency ratio of the plant.

Error bars in the graph do not overlap, so it shows that the mean photosynthetic ratios of tomato plants irrigated with Kızılırmak and tap water differ from each other. But this data does not show an exact and obvious difference. To make sure if there is a statistically significant difference between the mean photosynthetic ratios, a t-test was performed.

According to t-test- paired two samples for means, p-value (one tail) is found to be 2.19 x 10^{-6} which is smaller than the α value that is 0.05. This result causes H_0 to be rejected and H_1 is accepted. H_1 states that there is a statistically significant difference between the mean photosynthetic efficiency ratios of tomato plants irrigated with Kızılırmak and home water.

Plants demand particular heavy metals to help them to growth. However, excessive concentration of heavy metal can become noxious for plants and cause them to die. The capability of plants to acquire primary metals allows them to receive other nonessential metals. Since metals cannot be broken down, they sceptically influence the plant when concentrations top optimal levels. [10] Due to oxidative stress, repression of cytoplasmic enzymes and damage to cell structures take place. [11] ; another effect is that they displace fundamental nutrients at cation exchange localities of plants.

DISCUSSION

I was right in my hypothesis about decreasing photosynthesis rates. However, my guess was wrong about the number of tomatoes produced. The number of tomatoes produced in both plants did not have a great difference, the numbers were nearly equal and their appearances were the same. To understand the real reason, further researches are needed. My guess is that heavy metals didn't affect the number of tomatoes but other properties of the plant, such as the nutritious quality of tomatoes, but again I can't say anything specific about this situation since I only do my experimentation on chlorophyll levels.

Another thing I noticed was the colour of the plants, the experiment group was showing a great disparity in colour contrasted to the control group. The leaves of the experimental group were actually light-green, nearly yellow. In the introduction part, I talked about any decrease in germination parameters of plants developing on contaminated soils can be attributed to the diminished activity of photosynthesis, plant ore nourishment, and negatively affected the enzyme activity. [12] The decrease in chlorophyll concentrations was already apparent back then. My theory about the decrease in chlorophyll concentration was accurate. Even so, I wasn't presuming such a vast contraction.

The matter frightening me about this research is that the Kızılırmak river is also being used as drinking water even though it's visibly harmful to any living organism. I don't think I can drink water without checking its source from now on. If the contents of this Kızılırmak's water have such a harming effect on plants like that, who knows what is this water doing to us. My research is one of many pieces of evidence why this water should be stopped being used as drinking water and the cleaning and restoration activities must start in the Kızılırmak.

EVALUATION

I mentioned that the number of tomatoes in both plants did not have a great difference, the numbers were nearly equal and their looks were the same. Because of that, further investigation can be done to see the effects on agriculture and food production.

One of the strengths I have in that research is probably the temperature because tomatoes are warm and mild climate vegetables and die completely when the temperatures are below zero. The time I proceed with this experiment was the summertime and the climate was enough for seedlings to grow. Another strength is that the area I placed the pots was the outside. The reason for this is that tomato seedlings need strong direct light, the season characteristics also produced the direct light needed. Also, they need to move and sway in the breeze, to grow strong stems.

However, I also have limitations which I believe they may have created some insignificant or small errors. My first limitation was that I couldn't think how much they will grow in width. Because of this, after some time tomato plants became crowded in the pot, not crowded as much to block each other from getting sunlight or wind. In addition, the place of the leaves that were harvested might also affect the chlorophyll content recorded. The improvement I can suggest for this is to transplant tomato seedlings into their own individual 10 cm pots after 2 weeks. The second limitation may be the tap water. For the tap water, I don't know where it is coming from and so the contents of this water. The contents of the tap water may also be caused small harms to tomato seedlings. To gain better results and see the difference a bit more visible, the water may be purified in the laboratory beforehand.

Manufacturers create a tremendous product of waste which includes noxious chemicals and pollutants which can prompt air contamination and harm to us and our surroundings. Many of them do not have a decent waste management system and dump the waste in the fresh water which connects to rivers, canals and eventually to the sea. The toxic chemicals are also known to have an ability to change the appearance of water, increase the number of minerals, change the temperature of the water and posture a severe danger to organisms living in the water. [13] This investigation caused another research question to bear in my mind, which is about the type of metal ions that will elevate the growth of tomato plants. Since we cannot calculate the toxicity directly, we determine the effects of it. Plants can be used to determine the amounts of toxicity and if further researches take place, we can be able to determine what kind of chemical is responsible for the pollution. Because of this, I think the table in Appendix 1 is really useful to determine the changes in plants differing from the type of heavy metal. This table can be evaluated with further research and be used as a way to find out the pollution type or the cause of pollution at a small cost. This can be the topic of a further investigation arisen from this research about the chlorophyll content of tomato plants.

APPENDIX 1

Heavy metal	Plant	The toxic effect on plant
As	Rice	A decrease in germination; reduction in height of the seedling; decreased area of leaf
	Tomato	Reduced fruit yield; reduction in weight of leaf
	Canola	Stunted germination; wilting; chlorosis
Cd	Wheat	A decrease in germination; shrinkage in nutrient content of the plant; lessened root and shoot expanse
	Garlic	Lessened shoot enlargement; Cd gathering
	Maize	Lessened shoot development; inhibition of growth in roots
Co	Tomato	A decrease in the nutrient content of the plant
	Mung bean	Reduction in activities of antioxidant enzymes; a reduction in sugar, starch, amino acids, and protein content in plant
	Radish	A decrease in shoot expansion, root length, and leaf area; shrinkage in chlorophyll content; loss of nutrient content and reduced activity of antioxidant enzymes; a reduction in sugar, starch, amino acids, and protein content in plant
Cr	Wheat	Lessened root and shoot extension
	Tomato	Decrease in plant nutrient acquisition
	Onion	Inhibition of germination process; reduction of plant biomass
Cu	Bean	Collection of Cu in roots; root abnormality
	Black bindweed	Plant death; reduction in biomass and seed production

	Rhodes grass	A decrease in growth rate of roots
Hg	Rice	Reduction in height; yield reduction; bioaccumulation in seedlings
	Tomato	Reduction in germination; reduced height; decrease in fruit weight; chlorosis
Mn	Broad bean	Mn collection; reduction in shoot and root length; chlorosis
	Spearmint	Reduction in chlorophyll-a and carotenoid content; gathering of Mn in roots
	Pea	Reduction in chlorophyll a and b content; reduction in relative growth rate; reduced photosynthetic activity
	Tomato	Slower growth of plant; reduction of chlorophyll concentration
Ni	Pigeon pea	A decrease in chlorophyll content and stomatal conductance; decreased enzyme activity which affected Calvin cycle and CO2 fixation
	Ryegrass	Reduction in plant nutrient acquisition; decrease in shoot yield; chlorosis
	Wheat	Reduction in plant nutrient acquisition
	Rice	Inhibition of root growth
Pb	Maize	Reduction in germination percentage; suppressed growth; reduced plant biomass; a decrease in plant protein content
	Portia tree	Reduction in number of leaves and leaf area; reduced plant height; a decrease in plant biomass
	Oat	Inhibition of enzyme activity which affected CO2 fixation

Table 7. Effect of heavy metal toxicity on plants [14]

APPENDIX 2

The student has performed their experiment in Ankara Training and Research Hospital, Department of Biochemistry themselves with supervision from our staff.

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APPENDIX 3

Chlorophyll-a (mg/g1)	= 12.7 (A663) – 2.69 (A645) x VW
Chlorophyll-b (mg/g1)	= 22.9 (A645) – 4.86 (A663) x VW
Total chlorophyll concentration	= [20.2 (A645) – 8.02 (A663) x VW] / 1000
Photosynthesis efficiency ratio	= [12.7 (A663) – 2.69 (A645) x VW] / [22.9 (A645) – 4.86 (A663) x VW]

Table 8. The formulas of Lichtentaler and Wellburn to be used in the calculation of chlorophyll-a, chlorophyll-b and total chlorophyll levels [15]

A = absorbance at the given wavelength

W = the weight of the leaf sample

V = the final volume of the chlorophyll solution

<u>BIBLIOGRAPHY</u>

1. Subrahmanyam, N. S. and Sambamurty, A. V. S. S. (2000) Ecology, Narosa Publishing House.
2. https://www.greenpeace.org/archive-international/en/news/Blogs/makingwaves/world-water-day-10-devastating-facts/blog/44430/
3. Fergusson JE, editor. The Heavy Elements: Chemistry, Environmental Impact and Health Effects. Oxford: Pergamon Press; 1990.
4. He ZL, Yang XE, Stoffella PJ. Trace elements in agroecosystems and impacts on the environment. J Trace Elem Med Biol. 2005;19(2–3):125–140. [PubMed]
5. Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. Experientia supplementum (2012), 101, 133-64.
6. "Heavy metals" a meaningless term? (IUPAC Technical Report) John H. Duffus, Pure Appl. Chem., 2002, Vol. 74, No. 5, pp. 793-807
7. Sükran, D., Günes, T. and Sivac, R. 1998. Spectrophotometric Determination of Chlorophyll - A, B and Total Carotenoid Contents of Some Algae Species Using Different Solvents. Tr. J. of Botany. 22: 13±17.
8. https://www.hunker.com/12423700/the-effects-of-water-pollution-on-plant-growth
9. Lichtenthaler, H.K., Wellburn, A.R., Determination of Total Carotenoids and Chlorophylls A and B of Leaf in Different Solvents.
Biol. Soc. Trans. 11. 591-592 (1985).
10. Amujoyegbe, B.J.; Opabode, J.T.; Olayinka, A. (20 August 2007) African Journal of Biotechnology Vol. 6 (16), pp. 1869-1873. Obafemi Awolowo University, Ile-Ife, Nigeria
11. F. Assche and H. Clijsters, "Effects of metals on enzyme activity in plants," Plant, Cell and Environment, vol. 24, pp. 1–15, 1990.
12. White, P. J. (2012) Plant stress physiology. Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK.
13. Ioannis S. Arvanitoyannis. WASTE MANAGEMENT FOR THE FOOD INDUSTRIES Food Science and Technology International Series. University of Thessaly, Greece
14. G. U. Chibuike and S. C. Obiora, "Heavy Metal Polluted Soils: Effect on Plants and Bioremediation Methods," Applied and Environmental Soil Science, vol. 2014, Article ID 752708, 12 pages, 2014.
15. R. Djingova and I. Kuleff, "Instrumental techniques for trace analysis," in Trace Elements: Their Distribution and Effects in the Environment, J. P. Vernet, Ed., Elsevier, London, UK, 2000.