TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

International Baccalaureate

Biology Extended Essay

Effect of Different Fertilizers on Eutrophication

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Research Question: How does different types of fertilizers influence the replication rate of *Chlorella vulgaris*?

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Abstract

The aim of this experiment is to determine how nutrients in fertilizers affect the risk of eutrophication. My research question is: “How does different types of fertilizers influence the replication rate of Chlorella vulgaris?” Chlorella vulgaris is a microscopic alga that is found in fresh water. In order to observe this indicator, a counting method was used. Algae samples were administered with ammonium nitrate, diammonium phosphate, and compound fertilizers with 3 milligrams of a fertilizer in each sample, except for the control group. At the end of six days under ample light, samples (0.004 ml) were counted with a hemocytometer. ANOVA way applied to figure output whether therefore is a significant difference between mean values of algae number due to exposition to different fertilizers. The p-value was found to be 1.35E-9, which was smaller than the alpha value (0.05) therefore significant difference is proved. The results show that compound fertilizer has the greatest effect and ammonium nitrate has the least. The order in which the magnitude of their effects increases suggests that as the fertilizer contains a larger selection of nutrients, its effectiveness as well as the threat it poses to the nature in the context of eutrophication. In this experiment, compound fertilizer was found to be the most damaging fertilizer to the lake ecosystems. There was a control group in the experiment that shows how Chlorella vulgaris fares in plain water and if the fertilizers were in fact beneficial to their growth. There were on average 7350 cells per millilitre so all the fertilizers indeed improved the cell numbers.

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INTRODUCTION

With the growing population and depleting fossil fuel resources, conflicts in economy arises. Agriculture can be used to provide people with sustenance, or converted to biofuels to power vehicles or contribute to the power grid. Whether to invest utilize arable lands for food or foul is a debate of ethics. Nevertheless, both practices take place currently and increase the demand of efficiency from a given plot of land. This translates to increased usage of fertilizers in agricultural, which is usually done with inadequate knowledge and leads a great portion of the nutrients from the fertilizers will contaminate nearby lakes and rivers and trigger algal blooms.[2] Algal blooms presents risks to both surrounding habitats human health. Medicine and the living are my areas of interest so I found it fitting to study a subject in which anthropogenic activities extend back to humans themselves.

Interestingly, algae can also be used as biofuel without the disadvantage of occupying terrestrial land like other biofuel crops such as maize. Ongoing research on algal biofuel is increasing the efficiency of generating energy with algae. Algae have high prospect in that they are a very likely solution for an upcoming energy crisis in not so distant future. Current cultivation and harvesting costs limit the large scale use of algae and although they cannot power entire cities just jet, algal fuel has already began contributing in small portions. Algal fuel is also clean and renewable, hence almost ideal as an alternative energy source.[3]

Algae are photosynthetic organisms and are either unicellular or multicellular.[4] Despite being prokaryotes, cyanobacteria are sometimes referred to as algae. Unicellular or microscopic algae (including cyanobacteria) are called phytoplankton. Algae have cell walls. These cell walls may be composed of compounds other than saccharides. For example cell walls of diatoms are made up of silica which is facing threats of corrosion due to ocean acidification. Because algae are unicellular, and if they are multicellular they do not need

cellular differentiation, and their easy access to energy and biomass, they can grow and replicate faster than most organisms on earth. Arguably the fastest growing organism *Macrocystis pyrifera* (giant kelp) grows about 25-30 centimetres a day.[5]

As photosynthetic components of aquatic ecosystems, algae are responsible for initiating trophic chain, along with less productive chemosynthetic bacteria.[6] About 70 percent of the oxygen in the atmosphere is supplied from oceans.[7] Because phytoplankton enriched the atmosphere with oxygen, aerobic respiration became available and therefore biodiversity was enhanced.

Fertilizers are applied to increase the yield of the soil. Natural fertilizers are basically any dead tissue or organic waste while synthetic fertilizers are prepared using industrial methods. Natural fertilizers have lesser concentrations of nutrients compared to synthetic fertilizers mainly because they contain polymers which cannot be absorbed by the roots. Also nutrients are present in simpler forms in synthetic fertilizers, otherwise they would have to be decomposed before being available for the plant. In that matter, synthetic fertilizers are more efficient and faster than natural ones. Another use for synthetic fertilizers is to condition the soil and skip summer fallow, the act of leaving the ploughed land to rest for a few months.[8]

Fertilizers are oriented around three elements, macro nutrients which photosynthetic organisms need the most. Those are nitrogen (ammonia), phosphorus (phosphate) and potassium. Nitrogen is an essential part of chlorophyll.[9] It is necessary for every amino acid and nucleic acid. Phosphorus is found in nucleobases, ATP, and phospholipid bilayer. Among the three nutrients, nitrogen can be fixated by bacteria.[10]

For convenient distribution on large farmlands and rapid uptake by roots, farmers may prefer soluble variants of the fertilizers if available, such as Triple Super Phosphate (TSP).[11] The fertilizers are then solved in water and distributed via irrigation. However, because most of

the times the process is administrated poorly, excess fertilizers will travel to nearby lakes and streams through runoffs or leached into groundwater. Through sewers many other pollutants are dumped into surface waters such as detergents some of which contain phosphorus.[12]

This contamination may result with eutrophication which is a type of water pollution caused by nutrients. In this type of pollution algae plays a crucial role. During eutrophication phytoplankton replicate rapidly to form an algal bloom. Algal blooms can occur naturally in lakes and ponds but once enhanced by human factor, may cause discolouration in water due to photosynthetic pigments and deprive deeper levels of sunlight. Some portion of the algae dies continuously and descends upon the terrain. Aerobic bacteria then feed on the sunken algae and deplete oxygen in the process. During the day, oxygen levels in the water stay normal but at night photosynthesis stop and dissolved oxygen diminishes. As a results, surrounding water becomes an anoxic area, or a dead zone. Any aerobic organism caught with a dead zone will asphyxiate at once. Immobile creatures and crustaceans especially cannot outrun the anoxia. Not only dead zones destroy habitats for many aquatic species, but also intercept migratory routes for others.

Also some algal blooms or their accompanying predators may secrete toxins harmful for their surroundings and even humans. Such blooms are called harmful algal blooms (HABs). Toxics from HABs are usually neurotoxins. Some neurotoxins can accumulate in shellfish and poison humans following ingestion. Effects of the poison can be lethal depending on the amount.[13]

In light of these experiment, knowing the relation between the type of fertilizers and severity of eutrophication bears importance in regards to environmental pollution and the sustainability of ecosystems. In this context, the aim of this investigation to determine this relation and determine the most hazardous fertilizer. My research question is: “How does different types of fertilizers influence the replication rate of *Chlorella vulgaris*?”

According to an experiment[14], nitrogen is generally the limiting nutrient for phytoplankton. Some statistics from fao.org hints that photosynthetic life demands more nitrogen than phosphate or potassium. Therefore my hypotheses are

H0: there is no significant mean difference on *Chlorella vulgaris* replication rate due to the exposure with different types of fertilizers which are ammonium nitrate, diammonium phosphate and compound fertilizer.

H1: there is a significant mean difference on the *Chlorella vulgaris* replication rate due to the exposure with different types of fertilizers which are ammonium nitrate, diammonium phosphate and compound fertilizer.

METHOD

1) Method Development

The aim of this experiment is to deduce the impact of three commonly used synthetic fertilizers on the replication rate of green alga *Chlorella vulgaris*. The result will then be used to determine the most influential pollutant among the three due to eutrophication. My research question is: "What is the effect of different types of fertilizers on the eutrophication level indicated by *Chlorella vulgaris* count in fresh water? My hypothesis is that aquatic plants and algae will demand more nitrogen than phosphorus like their terrestrial counterparts.

My independent variable is the type of fertilizer alga culture is administered with. My dependent variable is the number of *C. vulgaris* cells after six days.

I chose to count the number of cells to determine the rate of replication which indicates eutrophication level. Use of fertilizers in agriculture accelerates the growth of plants. Since growth of cells for unicellular organisms should result in the acceleration of cell cycle, cells replicate more often in the presence of fertilizers. If fertilizers with different nutrient contents have also vary in their effect on the growth of *Chlorella vulgaris* cells, difference in the number of cell should produce viable data to compare the magnitude of the fertilizers' effect.

Diammonium phosphate and ammonium nitrate are among the most commonly used fertilizers in Turkey.[15] That makes the fertilizers both easier to procure and also a relevant threat to aquatic ecosystems. I tried to select fertilizers with different nutrients. For example, both urea and ammonium nitrate contain exclusively nitrogen so diammonium phosphate is a better alternative to urea as it contains phosphorus. The compound fertilizer is more balanced than others and contains the same amount of all three macronutrients. In this study these three fertilizers are used.[16]

The alga I used in this experiment is named *Chlorella vulgaris.* It is unicellular fresh water alga without a flagellum.[17] Because of these features, the cells are distributed evenly in the water with little effort. However, the cells are not suspended in water forever and clusters together and sink to the bottom. I have observed this situation while I was cultivating the initial sample. The samples were to be stirred before observed under the microscope in order not to overlook the additional biomass. *Chlorella vulgaris* has a high photosynthetic efficiency which makes it easier to cultivate in a shorter time interval. It was also one of the algae studied in a local university and was more accessible for the experiment.

Temperature was one of the controlled variables because it plays a critical role in metabolism of any organism. As the temperature affects the activity of enzymes, and photosynthesis involves several enzymes to be carried out, temperature should not differ between the samples if the fertilizers' effect alone is desired to be observed.

Intensity of the light incident on the samples was also critical. Since light is the only input of energy for *Chlorella vulgaris*, it dictates how much matter can potentially be put together and converted into organic biomass in the chloroplasts. Some of the energy also heats the system and change the temperature.

Wavelength of the light is also as critical as its intensity. Different wavelengths of light contain different energy levels. Also, chlorophylls found in the chloroplasts of *Chlorella vulgaris* do not absorb all wavelengths of light to same extent.

The amount of fertilizer in the samples may affect the results. There are different meanings regarding "amount" such as mass, volume or the number of molecules. In this experiment I chose to keep the mass constant because it was not only the easiest but also fertilizers are sold by their mass. Even the NPK value is actually the ratio of the elements to the compound by mass. Therefore each sample in the experiment **contains 0.5g of fe**rtilizer per litre.

Development of other organisms with fertilizers could be unpredictable. The native algae in the water may even outcompete *Chlorella vulgaris*. Although presence of a multitude of species in the water would make the model in the experiment resemble more of a natural ecosystem and explain the effect of the fertilizers on eutrophication more accurately, that still wouldn't answer the research question itself. Eliminating other species however has one perk, that only cells to be seen under a microscope are that of *Chlorella vulgaris* and not any other alga.

pH of the water is also influential in the activity of enzymes like temperature. However, pH could not be controlled as a variable because it was directly dependent on the chemical composition of fertilizers.

In order to control temperature, light intensity, and light frequency, all samples were cultivated in the same place and the same time so the temperature and weather conditions would be the same. The temperature is close to the room temperature, around 25°C. The glass tubes were aligned in a straight line in front of a window facing south and 10cm away from the glass. This ensures that they are all exposed to same intensity and wavelength of light, at the same angle and for the same duration.

There are several ways to measure the growth of algae in glass tubes, such as weighting the samples. Accumulation of biomass shows that more photosynthesis takes place than respiration. The two are reverse reactions, as seen in the formulas given below:

$6CO_2 + 6H_2O \rightarrow 6O_2 + C_6H_{12}O_6$ (Photosynthesis)

$6O_2 + C_6H_{12}O_6 \rightarrow 6CO_2 + 6H_2O$ (Respiration)

Gaseous reactants and products of these reactions are carbon dioxide and oxygen. Carbon dioxide is heavier than oxygen since both has two oxygen atoms but carbon dioxide has one

carbon atom in addition. If more photosynthesis takes place, more carbon dioxide will be absorbed than it is produced. Over time more and more carbon dioxide will dissolve in the water and eventually be fixated into biomass, the water will get heavier. Weighting the water with a sensitive device could detect this change. However evaporation will reduce the weight of the water as well and overall the strategy will be inconvenient to operate with.

Another method is to measure the oxygen gas released from the water. This can be done via probes and only in the presence of light. This method is unpreferable because rate of photosynthesis is incidental and slightest conditional inconsistency during measurement may result in incorrect conclusions from the experiment.

The easiest and most accurate method is to count the cells directly with a microscope. I used a hemocytometer, a special microscope slide with grid that divides the slide into regions with stated areas. The distance between the slide and its cover glass is 0.1mm, therefore the volume of any region can be calculated using the grid. Concentration of cells can then be found by dividing the number of cells to the selected volume in which they were counted. Liquids can be applied to the edge of the cover slip and adhesive forces will fill the empty space with the liquid. Hemocytometer was designed to count red blood cells and is still used widely for this purpose, due to its accuracy.[18]

In order to prevent contamination of the samples with other organisms, I boiled the water beforehand to destroy them with high temperature. While it is not the best sterilization method available, chemical sterilization on the other hand would also damage the algae. The samples were also vulnerable to contamination with airborne organisms.

Before the experiment, large amounts of *Chlorella vulgaris* was cultivated in a growth medium. In order to minimize the interference with the nutrients from the growth medium and lower the concentration of alga cells, I diluted the culture with more water. When finally

added to the test tubes, the sample was diluted by a factor of 606. The number of cells that could be counted with the cytometer was theoretically below 1, which means in water with the volume of 0.004mL, there might not be any cells at all. If the initial amount of the algae was higher, it would shadow to effects of the fertilizers.

2) Material List:

0.5g Ammonium Nitrate 33-0-0

0.5g Diammonium Phosphate 18-46-0

0.5g Compound Fertilizer 15-15-15

Glass Tubes 6mL × 20

Hemocytometer

Light Microscope (×100 magnification necessary)

Graduated Burette 5mL ±0.1mL

Beaker 1000mL ±1mL

Stirring Rod

Electric Kettle

3) Procedure

Measurements smaller than 5mL are done by the graduation scale on the burette. Larger volume measurements are done by the scale on the beaker.

1. To prepare initial ammonium nitrate solution, boil one litre of tap water.
2. Fill the beaker with the 1L tap water.
3. Add 0.5 grams of ammonium nitrate to the 1L tap water.
4. Stir with the rod until it dissolves completely.
5. Using the burette, fill 5 glass tubes with the ammonium nitrate solution, 5 millilitres in each tube.
6. Empty the beaker and clear it.
7. Repeat steps 1-6 with diammonium phosphate and then compound fertilizer instead of ammonium nitrate.
8. Boil 125 millilitres of tap water.
9. Fill the 1L beaker with the boiled 125 mL tap water.
10. Fill the remaining 5 tubes with the boiled tap water to be used as the control group. A total of 25mL is used.
11. Wait for remaining 100 ml of the boiled tap water to cool down for the purpose diluting *Chlorella vulgaris* culture.
12. When the beaker is cool to the touch, add 1mL of the *Chlorella vulgaris* culture to the beaker to dilute the alga culture.
13. Stir the water.
14. To figure out the initial number of *Chlorella vulgaris* in diluted culture, place the hemocytometer in the microscope.
15. Put its special cover slip on top of the hemocytometer.

16. Apply two drops of mixture with droplet from the beaker with diluted alga mixture to the edge of the coverslip.
17. Focus on a square that is made up of sixteen smaller squares.
18. Count the cells within the square, include the ones touching the upper and left lines and exclude the ones touching the lower and right lines.
19. Clear the hemocytometer and the cover slip and repeat 15-20 for 4 more times to be sure of the number of cells.
20. Fill 20 test tubes that have been filled with different fertilizers solutions 1 millilitre of the diluted alga culture. (number of cell per ….ml, development kısmına da neden bu miktarda seyrelttiğin)water (anlaşılmıyor).
21. Align the glass tubes in a line in front of a window facing south (in northern hemisphere), 10 centimetres away from the glass Tubes should not obscure each other.
22. Wait for six days.
23. After six days, to count the *Chlorella vulgaris* cells, place the hemocytometer in the microscope.
24. Put its special cover slip on top of the hemocytometer.
25. Take one of the test tubes that have ammonium nitrate solution and alga, gather 2 drops with the tip of the burette from this solution and apply them to the edge of the coverslip.
26. Focus on a square that is made up of sixteen smaller squares.
27. Count the cells within the square, include the ones touching the upper and left lines and exclude the ones touching the lower and right lines.
28. Clear the hemocytometer and the cover slip
29. Repeat steps 24-28 for the rest of the 4 tubes with ammonium nitrate and alga.
30. Repeat steps 26- 30 for 15 remaining test tubes.

ANALYSIS

1) Raw Data Table

Fertilizer	Trial	Number of Cells after 6 Days ±1/0.004ml	Temperature ±0.5 °C	Concentration of Fertilizer in each initial solution ±0.05g/L	Distance from the light source ±0.1cm	Number of Cells in Undiluted Alga Culture ±1/0.004mL	Name of the Studied Organism	Volume of Fertilizer Solution in Used in Each Trial ±0.1mL	Volume of Alga Culture Used in Each Trial ±0.1mL	Total Volume of Water ±0.2mL
AN	Trial 1	32	25.0	0.50	10.0	33	*Chlorella vulgaris*	5.0	1.0	6.0
	Trial 2	34								
	Trial 3	31								
	Trial 4	33								
	Trial 5	32								
DAP	Trial 1	35	25.0	0.50	10.0	33	*Chlorella vulgaris*	5.0	1.0	6.0
	Trial 2	37								
	Trial 3	33								
	Trial 4	34								
	Trial 5	36								
Compund	Trial 1	47	25.0	0.50	10.0	33	*Chlorella vulgaris*	5.0	1.0	6.0
	Trial 2	51								
	Trial 3	45								
	Trial 4	47								
	Trial 5	53								
Control	Trial 1	29	25.0	0.50	10.0	33	*Chlorella vulgaris*	5.0	1.0	6.0
	Trial 2	30								
	Trial 3	30								
	Trial 4	26								
	Trial 5	32								

Table1: Number of Cells per 0.004mL

Final number of cells in the alga culture after dilution was not counted because there were very few cells and sometimes there were no cells in the given volume at all.

2) Descriptive Statistics

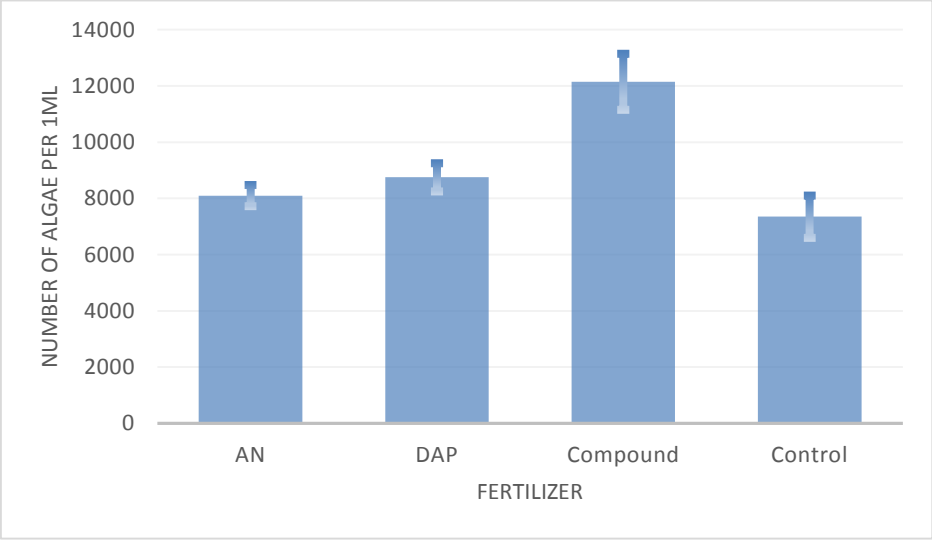
Number of cells were counted per 0.004mL. For ease of estimation, these values are multiplied by 250, the ratio of 1mL to 0.004mL. Number of cells in the alga culture are also divided by 606. This is because 1mL of the culture was diluted with 100mL of water, with a final volume of 101mL. 1mL from that mixture was then added to the test tubes with 5mL more water in them, further diluted by a factor of 6.

Fertilizer	Trial	Number of Cells after 6 Days ±250/ml	Number of Cells in Undiluted Alga Culture ±250/mL	Number of Cells in Diluted Alga Culture ±2.5/mL	Initial Number of Cells Test Tubes ±0/mL
AN	Trial 1	8000	8250	82	14
	Trial 2	8500			
	Trial 3	7750			
	Trial 4	8250			
	Trial 5	8000			
DAP	Trial 1	8750	8250	82	14
	Trial 2	9250			
	Trial 3	8250			
	Trial 4	8500			
	Trial 5	9000			
Compund	Trial 1	11750	8250	82	14
	Trial 2	12750			
	Trial 3	11250			
	Trial 4	11750			
	Trial 5	13250			
Control	Trial 1	7250	8250	82	14
	Trial 2	7500			
	Trial 3	7500			
	Trial 4	6500			
	Trial 5	8000			

Table2: Converted Data

Groups	*Count*	*Sum*	*Average*	*Variance*	*Standard Deviation*	*Range*
AN	5	40500	8100	81250	285	750
DAP	5	43750	8750	156250	395	1000
Compound	5	60750	12150	675000	822	2000
Control	5	36750	7350	300000	548	1500

Table3: Data Summary



Graph1: Fertilizers and Number of Cells per 1mL

3) ANOVA

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	67434375	3	22478125	74.15464	1.33E-09	3.238872
Within Groups	4850000	16	303125			
Total	72284375	19				

Table4: Analysis of Variance

DISCUSSION

Aim of this experiment is to assess the hazards of different fertilizers in regards to eutrophication. Accumulation of algal biomass in water is an indicator of eutrophication which in turn presents itself as an increase in number of cells, hence my research question is: "How does different types of fertilizers influence the replication rate of *Chlorella vulgaris*?"

I used the analysis of variance method because there were three independent variables. According to the analysis of variance of the cell counts from different samples, the *P-value* is 1.33E-9. Since the *P-value* is smaller than $\alpha = 0.05$, it can be said that there difference in the number of *Chlorella* vulgaris cells between groups cultivated in water treated with different fertilizers, or no fertilizer at all. Therefore the null hypothesis (H_0) is rejected and the alternative hypothesis (H_1) is confirmed, demonstrating that different fertilizers indeed affect the growth of the alga to different extents.

In the experiment, a control group was set that is meant to highlight the effect of fertilizers individually, to see if the fertilizer is in fact improving the growth of the algae. The control group contains no additional fertilizer but only the minerals that were already in the water. Since all other groups contain fertilizers with equal masses, the control group has been excluded from the analysis of variance in order not to interfere with the statistics as it infringes a controlled variable listed before: the amount of fertilizer. The research question focuses on "different fertilizers" which means that the control group is actually not a necessity but more of a convenience that enables a more objective approach to the collected data.

Because numbers of cells are small and are always integers, slightest of the changes result in relatively variances. Mean values are estimated in fractions to minimise the error by expanding the significant figures. Compound fertilizer displayed the greatest standard deviation with 822 cell (Table-2). Control group has a moderate deviation with 548 and DAP

and AN have lower deviations with 395 and 285 respectively. The results are nonetheless precise enough to provide substantial insight on the research question. Compound fertilizer has the greatest effect with a mean number of 12150 cells. Diammonium nitrate is the second in line with 8750 cells in average. The least effective fertilizer was ammonium nitrate with 8100 cells. All fertilizers had a positive effect on algae growth when compared to the control group with the lowest mean of 7350 cells. Overall it can be said that compound fertilizer is the fertilizer most damaging to the ecosystem. Although there are several other fertilizers untested by this experiment, these fertilizers are used frequently in agriculture at least in Turkey.

The results seem plausible because there is a pattern to the order of fertilizers' increasing effect. In the increasing order AN, DAP, and Compound fertilizer contains elements N, N-P, and N-P-K respectively. It is clearly seen that the fertilizer next in line contains one additional macronutrient than its predecessors. This suggests that the diversity of nutrients is of greater importance than quantity.

One curious element in the experiment is nitrogen. Due to the absence of nitrogen fixing cyanobacteria, the water could not reflect a true aquatic ecosystem. Since these bacteria produce the nitrogen that can be used by algae, the additional introduction nitrogen to the ecosystem displays a smaller impact in comparison. The nitrogen only fertilizer has the least contribution to the algae as it would happen in a lake or ocean so the experiment preserves its validity as its purpose is to draw conclusions and the statistics are simply means for that end.

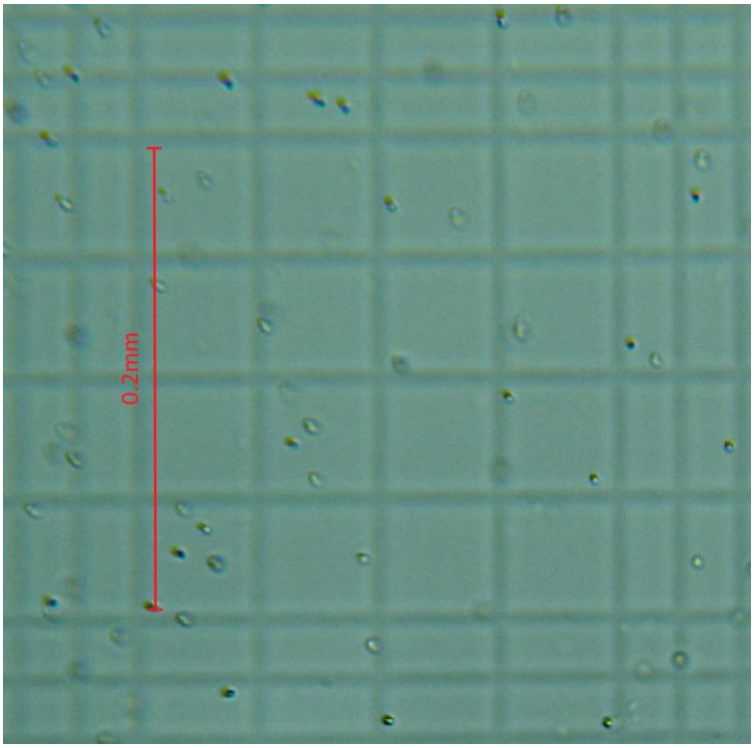
The answer to the research question is that fertilizers with diverse nutrient contents are more influential on *Chlorella vulgaris* growth and eutrophication. This means that in agricultural practices, greater care is required while dealing with fertilizers with two or more macronutrients, or else the risk of eutrophication rises.

The more convenient method for overcoming eutrophication is however, to control the fertilizer usage as a whole because if the excess fertilizer are found in water to cause eutrophication, then it is also removed from the farmlands and is actually a loss to the farmer. So the interests of nature and people should align on this aspect and a solution is made possible with substantial education and expert help.

Contents of the fertilizers travel from soil solved in water, so not all chemical species may travel in the same speed or quantity by runoff. In order to better mimic this phenomenon, a filtering mechanism can be implemented to the experiment. Soil can be used for this purpose although the composition of the soil is important, for example soil with high clay content filters the water well. Fertile soil types usually have similar characteristics and since these soil types are preferred for agriculture over barren lands, they are viable choices for the task as runoff from agricultural sites are usually filtered in these types of soil.

A terrarium with small animals, plants, and microorganisms can also make the experiment more accurate as it enables interactions that takes place in natural ecosystem which dictates both growth rate and carrying capacity for species. However that would need too much space and also foreign microorganisms would make it harder to detect and count *C. vulgaris* cells.

Appendices:



Chlorella vulgaris cells magnified a hundred times with a microscope. The square in the centre that is made up of sixteen smaller squares has edges of 0.2 millimetres, and an area of 0.04mm^2. The grid is separated from the cover slip with a distance 0.1mm. The total space has a volume of 0.004mm^3, which is the volume of water the cells are counted in. The cells in contact with upper and left edges are included in the count, those in contact with lower and right ones are excluded. The small orbs in the image are *Chlorella vulgaris* cells.

Image1: Hemocytometer under the Microscope

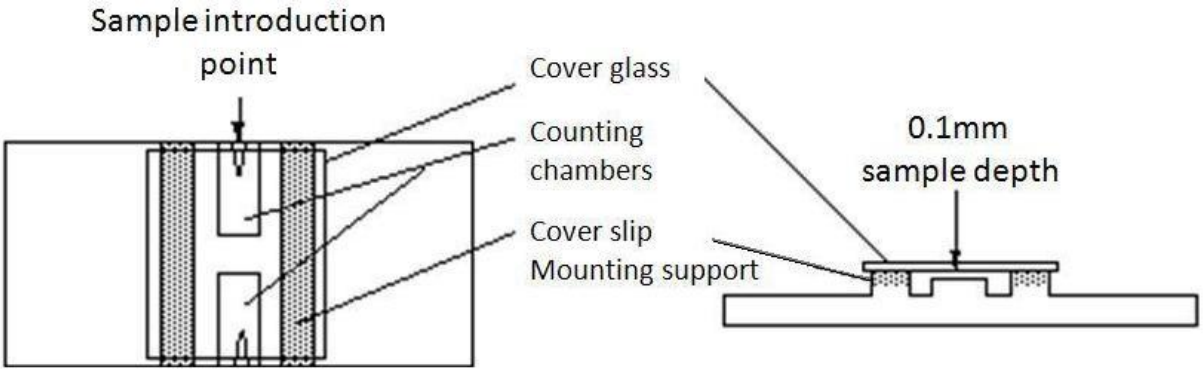


Image2: Structure of a Hemocytometer

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