Various Nitrogen-Based Fertilizer Applications and Their Influence on the Growth of Cyanobacteria

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I. Introduction

Cyanobacteria, commonly identified as blue-green algae, is notorious for multiplying exponentially, thus creating harmful algae blooms in nutrient-rich environments. These algae blooms prove to be calamitous in aquatic domains, depleting oxygen in bodies of water and blocking essential sunlight from reaching plants and animals, eventually creating dead zones in the ecosystem.

Fertilizer discharge has been pointed at as the main cause of cyanobacterial mass growth as inorganic fertilizer applications have taken over bodies of water. However, the global shift towards operating urea-based fertilizer, in an effort to lessen harmful effects on aquatic ecosystems rather than inorganic nitrogen fertilizers, has provoked a curious resurgence in cyanobacteria blooms. In this investigative experiment, I decided to compare the growth of an

isolated strain of living Spirulina Major, a form of cyanobacteria, when exposed to various kinds of nitrogen sources, specifically monoammonium phosphate and urea, and whether there is a significant difference.

II. Statistical Question: Is there a significant difference in the number of cyanobacteria cells present after the introduction to ammonium exposure and urea exposure?

Hypotheses:

H_O: $\mu_a - \mu_{ur} = 0$

H_A: $\mu_a - \mu_{ur} \neq 0$

Definitions:

μ_a = the true mean number of cyanobacteria cells produced when introduced to ammonium phosphate solution

μ_{ur} = the true mean number of cyanobacteria cells produced when introduced to urea-based solution

III. Data Collection

The basis of the experiment primarily consisted of data collection on the growth of Spirulina Major based on exposure from ammonium and urea fertilizer solutions to determine whether there is a statistical mean difference on the effectiveness of discrete nitrogen solutions. There were two treatment groups: one group with monoammonium phosphate concentration and another group with urea concentration.

First, I acquired 50 identical 10 mL vials with screwable lids and living Spirulina Major. I then inserted 1.5 mL of the Spirulina Major and 2 mL of distilled tap water into every vial using a 4 mL pipette. They were then deposited in a room without temperature fluctuations with a single source of light being from a window. Figure 1 depicts the set-up of the experiment,

including the identical measures of cyanobacteria and the nitrogen sources.

Figure 1. Set up of all the vials with respective solutions

Using the "hat method," a technique ensuring randomization to balance potential confounding, I wrote 25 1s and 25 2s on slips of paper. I placed them in the hat and randomly drew a slip of paper one at a time without replacement. If I pulled out a 1, I would add 1 mL of the ammonium solution to the vial and if I got a 2, I would add 1 mL of the urea solution. The process of making ammonium and urea solution initiated from the amalgamation of the 100 percent soluble powdered form of ammonium phosphate and urea and filtered water, proportionally a ratio of 1 teaspoon every 150 mL. After ensuring that 25 experimental units received the ammonium phosphate soluble solution and the other 25 units received the urea solution, I carefully separated them into two piles with monoammonium phosphate on the right side and urea solution on the left side. I then numbered the ammonium phosphate vials 1 through 25 and 26 to 50 for the urea containing vials to verify which vial has which solution. The 50 vials were placed 10 in a row and 5 in a column next to the windowsill, the location with sufficient sunlight.

After 14 days, I counted the cells to compare cell growth in order to validate whether urea solutions caused more algae growth. In order to precisely count the cyanobacteria cells, I used a hemocytometer to count the cells in the chamber. Using a 10 microliter pipette from OniLab, I placed the tip at the edge of the coverslip, allowing the sample to fill the chamber precisely. Figure 2 shows the microscope and hemocytometer set ups.

Figure 2. Set ups of the hemocytometer under the microscope and the OniLab microliter pipette filling the chamber.

Placing the hemocytometer under the microscope, the microscope focused on one set of squares on the very outside top right corner on the given counting chamber. After counting the cells in that one set, all four sets of 16 squares at all the corners have been counted and added up. While counting, the cells on the right and bottom sides of the chamber square are to be excluded. The same procedure was repeated for all fifty vials and the cells present in the 10 microliter injected to the hemocytometer grid were recorded. Figure 3 demonstrates a graphical grid model of how I counted all the 4 sets of the hemocytometer grid and a view from the microscope to show an accurate depiction of the cells present under the microscope.

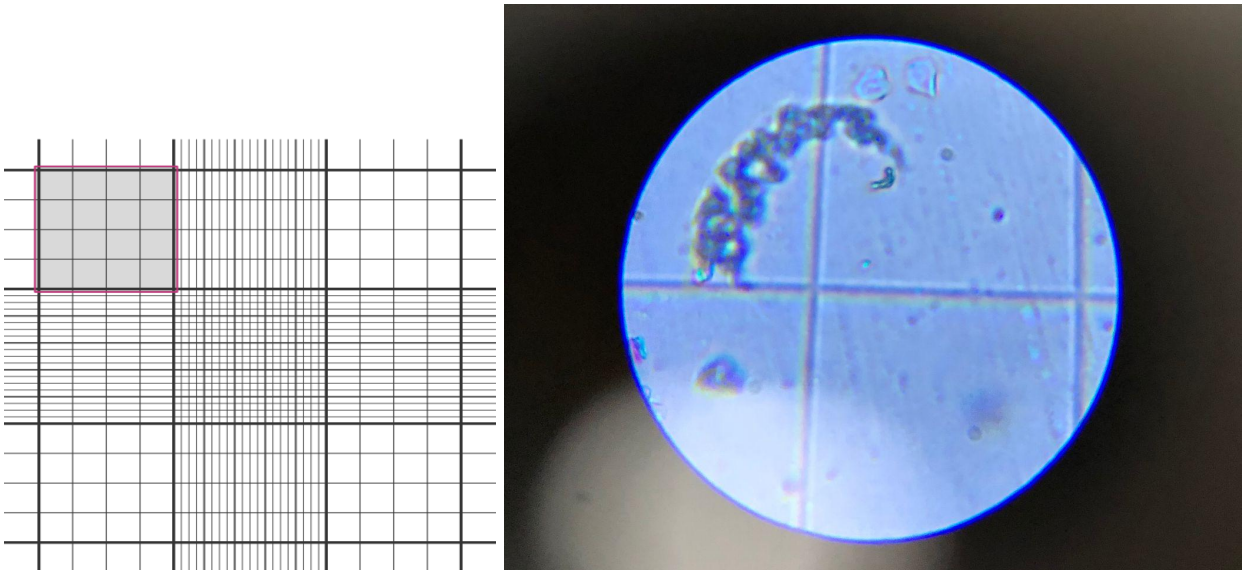

Figure 3. Hemocytometer counting chamber grid and a view from the microscope displaying cells from vial 5.

IV. Data Display

Raw Data:

Subjects	Ammonium treatment	Subjects	Urea treatment
Vial 1	124 cells	Vial 26	390 cells
Vial 2	149 cells	Vial 27	416 cells
Vial 3	175 cells	Vial 28	406 cells
Vial 4	134 cells	Vial 29	288 cells
Vial 5	112 cells	Vial 30	401 cells
Vial 6	94 cells	Vial 31	432 cells
Vial 7	155 cells	Vial 32	419 cells
Vial 8	219 cells	Vial 33	367 cells
Vial 9	122 cells	Vial 34	412 cells
Vial 10	138 cells	Vial 35	389 cells
Vial 11	136 cells	Vial 36	445 cells

Vial 12	117 cells	Vial 37	332 cells
Vial 13	115 cells	Vial 38	391 cells
Vial 14	143 cells	Vial 39	356 cells
Vial 15	102 cells	Vial 40	467 cells
Vial 16	133 cells	Vial 41	418 cells
Vial 17	129 cells	Vial 42	422 cells
Vial 18	218 cells	Vial 43	386 cells
Vial 19	147 cells	Vial 44	377 cells
Vial 20	131 cells	Vial 45	452 cells
Vial 21	115 cells	Vial 46	432 cells
Vial 22	98 cells	Vial 47	375 cells
Vial 23	152 cells	Vial 48	403 cells
Vial 24	127 cells	Vial 49	397 cells
Vial 25	136 cells	Vial 50	196 cells

Table 1. Raw data.

Summary Statistics:

Population	Population Mean	Sample Size	Sample Mean	Sample Standard deviation
a → ammonium treatment	μ_a	n_a=25	136.84	30.8
ur → urea-based treatment	μ_{ur}	n_{ur}=25	390.76	55.609

Table 2. The chart describes the summary statistics.

Boxplot Distributions:

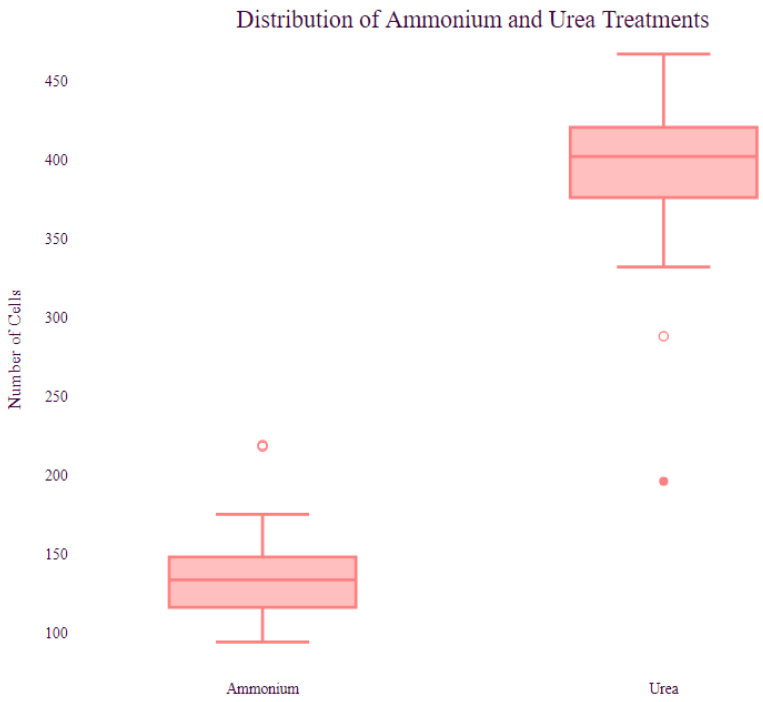

Figure 4. Boxplot of cell intensity of both ammonium and urea treatment. Notice how while the spreads are different and there are outliers, the shapes are similar because there is no apparent skew to the left or right. The IQRs(interquartile ranges) are approximately similar as well.

V. Data Analysis

Conditions for Tests of Inference:

1) Random: Same-sized vials with equivalent amounts of cyanobacteria and water amountage were randomly assigned to 2 treatments.
2) Independent: The result of one vial did not affect the results of other vials which means the samples were taken independently. This was applied to all jars in the experiment.
3) Normality: A sample of 25 was used for each of the 2 treatments (all together, there were 50 vials). Due to its relatively small sample size according to the Central Limit Theorem of sample sizes needing to be at least 30, I used the box-plot distributions to demonstrate the approximate normality in the shape of the graphs. Although they were not skewed,

the presence of outliers prevented the treatment results from being approximately normal: I must proceed with caution.

Calculations:

t-statistic	p-value	df	alpha level:α
-19.972	1.446×10^{-21}	37.458(from calculator)	$\alpha = 0.05$

VI. Conclusion

Because the p-value is $1.446 \times 10^{-21} < 0.05$, we reject H_O. There is sufficient evidence to claim that the true mean difference of the number of cyanobacteria cells between the ammonium phosphate and urea solutions (a-ur) is not as claimed. If there is no difference in the means, the probability of getting a value as or more extreme than ours is 1.446×10^{-21}, which is statistically significant as α level = 0.05.

VII. Reflection

Overall, my experiment process ran smoothly and it was extremely interesting seeing the growth of blue-green algae. While running my experiment, I was, however, constrained due to the lab environment and certain resource accessibility.

My experiment met several obstacles, first during the test subjects selection process. I originally wanted algae with pond water for my test subjects; however, due to inaccessibility and restraining orders, I was unable to incorporate algae typically exposed to nutrient discharge in the real-world environment. I instead bought Spirulina Major, a well-known type of cyanobacteria, with filtered water as my test subjects. Thus, there are limitations to my hypotheses because my treatments were only tested on Spirulina Major cyanobacteria, not various types of cyanobacteria. In addition, in taking into consideration the most cost-effective experiment with

holistic results, I used one type of monoammonium phosphate solution and one type of urea-based solution. I also only had 25 vials for each solution, thus being unable to ensure approximate randomization while checking my conditions. Further, my boxplot lineated outliers so I had to proceed with caution during my experiment. Another major challenge that was faced was the struggle to constrain bias from my lab environment. I set up the lab in my room next to a sunlit window but due to the number of jars, I suspect that some jars did receive more sunlight than others. This could potentially provide bias as sunlight and warm temperature are also prominent factors that expedite cyanobacteria growth. However, this was a bias I predicted beforehand and the utilization of randomization and replication, 2 of the 3 basic principles of experimental design, served to lessen this potential bias. By randomly assigning the two treatments (either ammonium or urea), the treatment groups were equalized as the effects of lurking variables were balanced out. Further, replication ensured the alleviation of detecting differences in the effects of the two treatments.

For future applications, experimenting with various cyanobacteria instead of being restricted to one type could be conducted additionally. This experiment did lack absolute authenticity because I utilized bought algae and one type of ammonium and urea fertilizer solutions. Moreover, future experiments should definitely have at least 30 vials per solution to meet the conditions for approximate normalization. A more realistic experiment would have acknowledged the incorporation of both a control group and levels of solution concentration and thus embedded different types of ammonium solutions as well as different types of urea solutions.

VIII. Works Cited

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