Seed germination in response to pH: Effect of tap water, alkaline water, and carbonated water on *Phaseolus lunatus*

Vanessa Chow, Julia Shim & Shannon Lo

Abstract

This study aims to determine which type of water and what pH range is most optimal for germinating seeds of *Phaseolus lunatus,* as there have been limited studies focused on the pH of water. Seeds of *P. lunatus* were germinated under three separate water treatment groups with varying pH values. In this particular study, we used tap water from British Columbia's lower mainland, alkaline water, and carbonated water. *P. lunatus* root growth was observed over the span of two weeks, where we considered a longer root length to be associated with higher germination success. We calculated a mean root length using the compiled data, which included each group member's measurements. *P. lunatus* germinated in a tap water environment had the longest mean root length of 9.83 centimeters; however, using a Kruskal-Wallis test, these results were not found to be statistically significant ($p= 0.9049$). With this, we conclude that none of the water treatments yielded *P. lunatus* root lengths that were statistically greater in length compared to *P. lunatus* grown in other conditions.

Keywords: water, pH, acidic, alkaline, germination, *Phaseolus lunatus*, lima beans

Introduction

pH is determined based on the acidity or basicity of water with a range from 0 to 14 (USGS, n.d.). At standard state temperature, a pH of 7 is neutral, while a pH above 7 is a base and a pH below 7 is an acid (USGS, n.d.). Overall, pH can indicate the quality of water and can be harmful when changed from drivers like pollution and climate change (USGS, n.d.; Jiao et al., 2016). For example, according to Lovejoy (2018), soil pH is important for effective nutrient uptake as very alkaline or very acidic soils can inhibit micro or macronutrient availability for plants. However, soil pH has increased with global warming which tends to negatively affect soil nutrients such as carbon, nitrogen and phosphorus (Jiao et al., 2016). Overall, soil pH and climatic variables are well correlated with various nutrients, which could have detrimental effects on plant growth (Jiao et al., 2016).

In a study by Gentili et al. (2018), *Ambrosia artemisiifolia* was grown in soil at pH values of 5, 6 and 7, but grew best at a slightly acidic pH of 6. Similarly, *P. lunatus* typically grows most effectively at a soil pH range between 6.0−6.8 (Baudoin, 2006). However, in terms of the pH of water, the germination success of seeds for most species is generally negatively affected at high pH levels due to a high quantity of nitrogen-containing compounds (Pérez-Fernández et al., 2013). While previous studies have been conducted in regards to the effect of soil pH as well as temperature and salinity on the growth of *P. lunatus,* there has been limited research on how the pH of the water directly affects the germination of *P. lunatus* (Pollock, 1969; Ballhorn et al., 2018; Pérez-Fernández et al., 2013). With this information, we were motivated to determine if seeds germinated in a more acidic environment would fare better than those germinated in alkaline conditions by using different types of water ranging in varying pH values.

Our study focuses primarily on determining how different types of water with different pH levels affect the germination success of seeds of *P. lunatus*. Therefore, using *P. lunatus* as our experimental unit, we investigated each bean's root growth when germinated using tap water from British Columbia's lower mainland with a pH of approximately 7.5, Icelandic Glacial alkaline water with a reported pH of 8.4, and San Pellegrino carbonated water with an estimated pH of 5.3 (Alcademics, 2013). However, after testing each water treatment with purple cabbage pH strips, we determined the tap water to have a pH of 6−7, alkaline water to have a pH of 7−8, and carbonated water to have a pH of 8−9. We then hypothesized that if a lower pH around 6.0−6.8 allows *P. lunatus* to grow most effectively, then the tap water with a pH between 6 and 7 will most likely grow *P. lunatus* with longer roots than those grown with alkaline or carbonated water.

Methods

We investigated the mean final root length of *P. lunatus* after two weeks of germination when exposed to three separate water treatments. Given that all three group members are located in the lower mainland area of British Columbia (B.C.), one of our water treatments was tap water, which has an estimated pH of 6−7. The second water type we used was bottled alkaline Icelandic Glacial Water, which has a pH of 7−8. Finally, we used bottled San Pellegrino carbonated water with a pH of 8−9.

We dampened three sheets of paper towel, each with 5 mL of a different water type (tap water, alkaline water, and carbonated water) using a measuring spoon. Next, we placed five dry lima beans between each paper towel and folded the paper towel over the beans, for a total of $n= 15$ lima beans per group member to observe and measure over the span of two weeks. There were initially $n= 45$ lima beans for our overall study. Next, the beans were placed into three separate Ziploc bags, one for each water treatment. For each bag, we numbered *P. lunatus* from one to five such that each bean's growth could be consistently monitored over our growing period. Every two days, we added 5 mL of water to each paper towel using the respective water treatment to maintain an appropriate level of dampness. We then recorded the root growth of each *P. lunatus* over a two-week period with measurements recorded every second day to determine the root growth of *P. lunatus* over time. To measure the roots, we used a string to account for all root shapes, such as bent or curled roots. We then measured the string with a ruler, which was recorded every other day for each bean. After the two-week period, we compiled the final root lengths of all *P. lunatus* from each group member. Any lima beans that displayed some form of decay—such as mold—over our observation period was excluded from the final data. At the end of the two-week period, nine *P. lunatus* were discarded from our experiment, and not taken into account in our analysis.

Statistical Methods

A total of $n= 36$ lima beans were assessed after omitting any decaying *P. lunatus* from each group member. After selecting only healthy individuals, the tap water treatment, alkaline water treatment, and carbonated water treatment had a sample size of $n= 12$, $n= 11$, and $n= 13$, respectively. One-way Brown-Forsythe and Welch ANOVA tests were conducted using Prism GraphPad (version 9.0.0) to determine if each of the three water treatments of different pH values grew roots of similar lengths. However, from the QQ plot, homodestacicity plot, and residual plot, we determined that our dataset did not meet the required normality assumptions for a one-way ANOVA test. As a result, we decided to conduct a nonparametric Kruksal-Wallis test to assess for any significant differences between the root lengths of the lima beans in each water treatment.

Results

Figure 1 demonstrates the mean root length of *P. lunatus* in tap water (9.83 cm), alkaline water (7.92 cm), and carbonated water (8.42 cm), with error bars representing 95% confidence intervals. This indicates that over a two-week period, the alkaline water yielded the lowest average root length, while the tap water yielded the greatest mean root length (Figure 1). Our chosen significance level is $\alpha= 0.05$, where we accept a 5% risk of rejecting a true null hypothesis where there is no difference in mean root length between water treatment groups. We chose this alpha value of $\alpha= 0.05$ as it is the standard in biological sciences. Additionally, our

Kruskal-Wallis test using Prism GraphPad (version 9.0.0) produced a p-value of 0.9049 ($p > 0.05$), indicating that our results are not statistically significant.

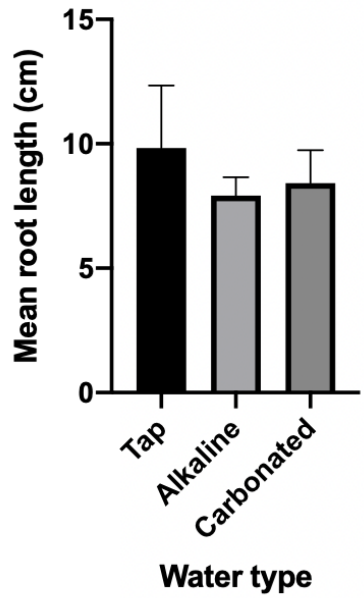

Figure 1. *Mean root length of P. lunatus from each water treatment group after 2 weeks of germination.* Bars represent the mean root length (in centimeters) of *P. lunatus* from the tap water treatment group (*n= 12*), alkaline water treatment group (*n= 11*) and carbonated water treatment group (*n= 13*), with error bars indicating 95% confidence intervals. The mean root length of *P. lunatus* from the tap water, alkaline water, and carbonated water treatment groups is 9.83 centimeters, 7.92 centimeters, and 8.42 centimeters respectively. A nonparametric Kruksal-Wallis test yielded a p-value of p= 0.9049.

Discussion

Since *P. lunatus* is reported to grow best in an environment with a soil pH of 6.0−6.8, we hypothesized that in our experiment, *P. lunatus* would germinate best when treated with tap water (Baudoin, 2006). We predicted this because tap water presented to have a pH range of 6−7, which has the closest pH range as the ideal pH for *P. lunatus* growth. In contrast, alkaline water has a pH range of 7−8, while carbonated water has a pH range of 8−9. Due to the similar pH ranges of our tap water and the reported ideal soil pH for growth, we hypothesized that tap water would yield the greatest *P. lunatus* germination success. As we attained a p-value of 0.9049 (p >

0.05) from our nonparametric Kruskal-Wallis test, we fail to reject our null hypothesis and conclude that there is no statistically significant difference in the germination success of *P. lunatus* grown in various water conditions.

P. lunatus grows at an ideal pH range of 6.0−6.8 (Baudoin, 2006). This is because when placed in an acidic environment with a pH value less than 6.0, the essential nutrients for growth—such as nitrogen, phosphorus, and potassium—may not be readily available (Jensen, 2010). Similarly, in an alkaline environment with a pH value greater than 7.5, some essential nutrients for growth—such as iron, manganese, and phosphorus—may also not be available to help the plant grow healthily (Jensen, 2010). For most plants, a neutral pH between 6.5−7.5 is most ideal for growth, as it is the optimal range for plant root growth (Jensen, 2010). Therefore, maintaining a neutral soil pH environment will allow for *P. lunatus* to absorb the essential nutrients required for growth.

Our results indicated that tap water yielded the greatest lima bean growth. This aligned with our hypothesis and literature, in that the most ideal pH for lima bean growth is between 6.0−6.8. These results were most likely due to there being a significant lack of essential nutrients in the alkaline water and the carbonated water. Many micronutrients are unavailable in an alkaline soil pH that is greater than 7.5, while they are present at slightly more acidic pH levels such as 6.5 (Jensen, 2010). The Icelandic Glacial alkaline water that we used contained calcium, magnesium, and sodium (Icelandic Glacial, n.d.). However, these were not some of the nutrients that would be essential for *P. lunatus* growth, as outlined above. This aligns with our results, as the growth of *P. lunatus* treated with alkaline water displayed the shortest growth in comparison to the other two water types. While we were only able to test the germination success of *P. lunatus* in the water environments rather than soil, we likely would have seen a similar trend after transferring the species to soil as the water in the soil would be more alkaline and hence lack some of the essential nutrients necessary for *P. lunatus* germination.

We predicted that the San Pellegrino carbonated water would be the most acidic, as CO_2 becomes a carbonic acid when dissolved in water, thereby lowering the pH (Britannica, n.d.).

$$CO_{2\ \text{(carbon dioxide)}} + H_2O_{\text{(water)}} \rightleftharpoons H_2CO_{3\ \text{(carbonic acid)}}$$

However, using our pH strips, we determined the pH value of the carbonated water to be 8–9, which is much greater compared to the other two water types. This was most likely due to the San Pellegrino brand adding far less CO_2 compared to other brands of carbonated water in order to keep the bubbles lighter and smaller, which makes this brand of water have a moderate amount of carbonation relative to other brands (Bonne O, n.d.). However, because the amount of carbon dioxide concentration plays a role in the acidity of the water, having a lower amount of CO_2 would result in a less acidic water. When adding water every other day to the lima beans, the acidity of the carbonated water most likely fluctuated. This is because atmospheric CO_2 dissolves into the water over time, making the water more acidic as time goes on (Britannica, n.d.). For future studies, choosing a different carbonated water—such as Perrier—that has an acidic pH would be beneficial to observe the effect that an acidic environment has on the growth of *P. lunatus*. This is because the plant is able to withstand soils with a pH as acidic as 4.4, and hence may produce significant results in growth differences (Baudoin, 2006).

Sources of Error

Given that our results were not statistically significant ($p > 0.05$), there is insufficient evidence to conclude that one of the three water treatments (out of tap water, alkaline water and carbonated water) yielded *P. lunatus* with greater root lengths compared to the others. Therefore, we are unable to lend support to the fact that pH does in fact have an effect on the germination success of *P. lunatus*. Extraneous variables that may explain our results include errors in consistency across each of our group members' experiments, as well as failing to include a large enough sample size. According to Baudoin (2006), although *P. lunatus* can be grown in a wide range of ecological conditions, they fare best in humid environments at low altitudes. However, these conditions could not be replicated as this study was conducted in our homes as opposed to in a controlled lab environment at the university. Further, without access to a lab, other environmental factors such as temperature and sunlight were also inconsistent across each of our experimental setups. Although we had a total of $n = 45$ individuals to begin with, some *P. lunatus* began to decay, and therefore had to be discarded from our study. After omitting these individuals, we had $n = 36$ individuals. In the future, a similar experiment could be conducted where all *P. lunatus* are germinated in the same location, such that all other conditions aside from the pH of the water treatment are held constant. This experiment could also be repeated with a greater starting number of individuals to ensure that even after omitting any unhealthy *P. lunatus,* there is still a sufficiently large sample size.

Conclusion

Three separate water treatments with varying pH values (tap water, alkaline water and carbonated water) were used to germinate seeds of *P. lunatus*, and the lengths of the roots were compared after two weeks of growth by three group members. None of the three water treatments produced *P. lunatus* with roots statistically longer in comparison to the others. It was initially hypothesized that *P. lunatus* treated with tap water would experience optimal germination in comparison to individuals treated with alkaline and carbonated water. However, while our data suggests that *P. lunatus* treated with tap water did in fact grow longer roots than *P. lunatus* germinated in both alkaline and carbonated water environments, upon analyzing our data using a nonparametric Kruksal-Wallis test, these results were not statistically significant ($p > 0.05$). Our failure to obtain statistically significant results may be a result of various extraneous variables such as different environments provided by each group member.

Acknowledgements

We would like to thank our professor, Dr. Celeste Leander, and the teaching team of BIOL 342 for providing us with guidance throughout the study. We would also like to acknowledge UBC for giving us the opportunity to conduct this study and learn through this integrative biology laboratory. Lastly, we would like to acknowledge that UBC's Vancouver Point Grey campus is situated on the traditional, ancestral, unceded territory of the Musqueam people.

References

Alcademics. (2013). Measuring the pH of mineral waters. *Alcademics.* https://www.alcademics.com/2013/04/measuring-ph-of-mineral-waters.html

Ballhorn, D. J., Wolfe, E. R., Tyler, J., Ronan, W., Sands-Gouner, S., Shaw, C., ... & Kautz, S. (2018). Quantitative effects of soil salinity on the symbiosis of wild lima bean (Phaseolus lunatus L.) and Bradyrhizobium in Costa Rica. *Journal of Applied Botany and Food Quality*, 91, 304-309.

Baudoin, J.P. (2006). Plant Resources of Tropical Africa 1: Cereals and pulses. *Prota Foundation*.

Bonne O (n.d.). The Science of Carbonation: A visual guide to great carbonation. *BonneO*. https://bonneo.ca/blogs/our-creations/18626521-the-science-of-carbonation-a-visual-guide-to-great-carbonation

Gentili, R., Ambrosini, R., Montagnani, C., Caronni, S., & Citterio, S. (2018). Effect of Soil pH on the Growth, Reproductive Investment and Pollen Allergenicity of *Ambrosia artemisiifolia* L. *Frontiers in plant science*, *9*, 1335. https://doi.org/10.3389/fpls.2018.01335

Icelandic Glacial. (n.d.). FAQ. *Icelandic Glacial.* https://icelandicglacial.com/pages/faq#:~:text=What%20minerals%20or%20additives%20are,nor%20added%20to%20the%20water

Jensen, T.L. (2010) Soil pH and the Availability of Plant Nutrients. *IPNI Plant Nutrition TODAY*, 2.

Jiao, F., Shi, X. R., Han, F. P., & Yuan, Z. Y. (2016). Increasing aridity, temperature and soil pH induce soil C-N-P imbalance in grasslands. *Scientific reports*, *6*, 19601. https://doi.org/10.1038/srep19601

Lovejoy, R. (2018). How does ph affect plants?. *SFGATE*. https://homeguides.sfgate.com/ph-affect-plants-49986.html

Pérez-Fernández, M. A., Calvo-Magro, E., Montanero-Fernández, J., & Oyola-Velasco, J.A. (2013) Seed Germination in Response to Chemicals: Effect of Nitrogen and pH in the Media. *Journal of Environmental Biology*, 27(1), 13-20.

Pollock B. M. (1969). Imbibition temperature sensitivity of lima bean seeds controlled by initial seed moisture. *Plant physiology*, *44*(6), 907–911. https://doi.org/10.1104/pp.44.6.907

The Editors of Encyclopaedia Britannica. (2020). Carbonic acid. *Encyclopedia Britannica*. https://www.britannica.com/science/carbonic-acid

USGS. (n.d.). pH and Water. *USGS Science for a changing world*. https://www.usgs.gov/special-topic/water-science-school/science/ph-and-water?qt-science_center_objects=0#

APPENDIX

Vanessa's Raw Data

	Date: Mar. 13/21	Date: Mar. 15/21	Date: Mar. 17/21	Date: Mar. 19/21	Date: Mar. 21/21	Date: Mar. 23/21	Date: Mar. 25/21
Lima bean sample	Root length (cm)	Root length (cm)	Root length (cm)	Root length (cm)	Root length (cm)	Root length (cm)	Root length (cm)
Tap water 1	0	0	0	2.5	6.6	12.4	16.7
Tap water 2	0	1.8	2.4	3.8	7.2	9.4	12.2
Tap water 3	0	2.3	2.8	4.1	7.8	13.4	14.6
Tap water 4	0	2.4	2.6	5.3	6.7	8.8	11.4
Tap water 5	0	2.3	2.6	5.4	8.8	12.7	15.3
San Pellegrino 1	0	0	0.1	0.6	3.4	5.1	6.2
San Pellegrino 2	0	0	0.2	1.4	4.6	6.8	8.3
San Pellegrino 3	0	0	0	2.2	5.7	7.4	9.1
San Pellegrino 4	0	0	0.2	1.4	4.2	5.8	7.2
San Pellegrino 5	0	0	0.5	1.3	4.5	5.1	5.6
Icelandic 1	0	0	0.4	1.8	x	x	x
Icelandic 2	0	0.3	0.8	2.2	5.1	6.6	7.4
Icelandic 3	0	0.2	0.5	1.4	3.3	4.1	5.3
Icelandic 4	0	0.7	1.4	3.2	5.1	6.2	6.8
Icelandic 5	0	0.8	1.2	2.6	2.9	x	x

Shannon's Raw Data

date	TAP water					ALKALINE (icelandic glacial water)					CARBONATED (san pellegrino)				
	#1	#2	#3	#4	#5	#1	#2	#3	#4	#5	#1	#2	#3	#4	#5
Mar 9/21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mar 10/21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mar 11/21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mar 12/21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mar 14/21	0	0	0	0	0.5	0.5	1.0	1.0	1.0	1.0	0	0	0	0	1
Mar 15/21	0	1.0	1.0	0	1.0	1.0	1.5	1.5	2.0	1.5	0	0	0	1.0	1.5
Mar 16/21	2.0	2.0	2.5	X	3.0	2.0	2.5	3.5	4.0	3.0	0.5	2.0	0.5	3.5	3.5
Mar 17/21	2.6	2.5	2.8	X	3.0	2.5	2.5	3.7	4.0	3.7	0.5	3.0	0.5	5.0	3.8
Mar 18/21	3.0	3.0	3.0	X	3.4	3.2	3.9	4.2	5.2	3.8	0.5	3.4	0.5	6.6	4.8
Mar 19/21	4.5	4.6	3.5	X	4.7	5.3	5.5	5.5	6.1	5.0	0.5	3.5	1.0	7.0	5.9
Mar 20/21	5.0	5.5	3.9	X	5.5	6.0	6.1	5.5	6.2	5.0	0.5	4.3	1.0	9.8	6.5
Mar 21/21	5.0	6.0	3.9	X	6.0	8.0	8.4	6.5	7.3	5.0	0.5	6.0	1.0	11.8	8.0
Mar 23/21	7.0	6.7	4.9	X	6.3	8.2	9.4	8.0	8.4	5.0	0.5	6.3	1.0	14.4	9.9

Julia's Raw Data

Tap Water

Day	#1	#2	#3	#4	#5
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0.7	0.5	0	0	0
6	1.5	0.7	0.5	0	0.6
7	2.3	1.5	1.1	0.5	1.3
8	3.4	2.3	1.5	1.0	1.4
9	4.5	3.4	2.4	1.4	2.3
10	5.1	4.5	3.3	1.9	3.5
11	5.7	5.1	4.4	2.3	4.5
12	6.3	5.7	5.5	2.9	5.3
13	6.8	6.3	6.1	3.4	5.9
14	7.3	7.0	6.8	X	6.7

Alkaline Water

Day	#1	#2	#3	#4	#5
1	0	0	0	0	0

2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0.5	0	0	0.3	0
6	1.3	0	0.7	0.9	0.5
7	2.5	0.5	1.4	1.6	1.2
8	3.4	1.0	2.8	2.5	2.5
9	4.9	1.4	3.5	3.3	3.2
10	5.6	1.9	4.7	4.6	4.4
11	6.8	2.3	5.5	5.3	5.6
12	7.9	2.9	7.0	6.4	6.5
13	8.2	3.3	7.6	7.5	7.4
14	8.5	X	8.2	8.6	8.3

Carbonated Water

Day	**#1**	**#2**	**#3**	**#4**	**#5**
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	1.0	0.3	0	0.5	0.3
6	1.5	0.9	0.5	1.2	1.0
7	3.7	1.7	1.3	2.4	2.2
8	3.9	2.3	2.0	3.7	3.5

9	4.9	3.2	2.8	4.3	4.2
10	5.7	4.9	3.6	5.8	5.4
11	6.8	5.6	4.7	6.2	6.2
12	7.3	7.0	6.0	7.5	7.6
13	7.9	7.8	7.1	8.0	8.1
14	8.7	8.5	8.2	8.6	8.5

Compiled Data

Tap water	Alkaline	Carbonated
7.0	8.2	6.3
6.7	9.4	14.4
6.3	8.0	9.9
16.7	8.4	6.2
12.2	7.4	8.3
14.6	5.3	9.1
11.4	6.8	7.2
15.3	8.5	5.6
7.3	8.2	8.7
7.0	8.6	8.5
6.8	8.3	8.2
6.7		8.6
		8.5

Figure 2. *Compiled data showing the final root lengths of P. lunatus from each water treatment group after two weeks.* After discarding any individuals with signs of decaying (such as mold), the sample size of the tap water group, alkaline water group, and carbonated water group is *n= 12, n= 11, n= 13,* respectively.