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Modeling the effects of acid rain on bacterial growth

Divyash Shah, Katherine McCormack
Tenafly Middle School, Tenafly New Jersey

SUMMARY

Acid rain has caused devastating decreases in ecosystems across the globe. This problem has been escalating due to the burning of fossil fuels. To mimic the effect of acid rain on the environment, we analyzed the growth of gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus epidermidis*) bacteria in agar solutions with different pH levels. We tested the hypothesis that agar with higher concentrations of vinegar would inhibit growth of gram-negative and gram-positive bacteria. We used agar with no bacteria as the negative control to ensure that there was no contamination of the agar from the environment. After 7 days at 25°C, we measured the size and number of the bacterial colonies. Our results showed that there was growth of both bacteria in each of the different agar concentrations, while the control agar exhibited no growth. The growth of the *Escherichia coli* (*E. coli*) colony was much greater than from the Staphylococcus epidermidis (*S. epidermidis*) colony at 3 different concentrations of vinegar: 6.25% Vinegar, 12.5% Vinegar, and 25% Vinegar. 6.25% Vinegar models current conditions in lakes while 25% represents future models at the rate we are going. While both bacteria grew in all solutions, there was clear evidence that more growth was visible in the agar with less vinegar. Also, measurement of the growth of each bacterium with daily check-ups over the week-long study showed the bacteria grew quicker in the agar with no vinegar. These results show that in a given acidic environment there was a significant decrease in bacterial growth with an increase in vinegar concentration in the agar, suggesting that bacterial growth is impacted by the pH of the environment. Therefore, increased levels of acid rain could potentially harm the ecosystem by altering bacterial growth.

INTRODUCTION

Humanity's footprint on the world has caused the influx of gases in our atmosphere. Gases, like sulfur dioxide (SO_2) and nitrogen oxides (NO_x), enter the atmosphere, where they mix with water (H_2O) deposits and form acidic liquids (1). During precipitation, these acidic liquids can cause damage to populations of species as well as their environments. Acid rain is often forgotten as a damaging consequence of the burning of fossil fuels (2).

The potential dangers of acid rain include damaging vegetation, destroying aquatic food chains, and polluting lakes and rivers, among many other bodies of water (1). Acid rain, also known as acid deposition, refers to any source of precipitation that includes acid, mostly SO_2 and NO_x, that falls to the ground in either wet or dry deposits. The United States Environmental Protection Agency declares that the primary causes of acid rain include the burning of fossil fuels, emissions from automobiles and other vehicles, and byproducts from labor industries, including manufacturing and oil refineries (3). Though human involvement is the key reason for increased acid rain in the 21st century, there have been natural causes for the release of SO_2 and NO_x, including volcanic explosions, lightning, and decaying biological life (4). The combination of man-made and natural releases of these gases causes a disastrous impact. Natural water has a pH of 6.5 – 7.0, while acidic rain has a pH of 4.4, well below the normal levels for our biosphere or the animals in it (5).

The consequences of acid rain include major damage to both water and land ecosystems on the surface of the earth (3). When these dissolved gases fall to the ground, the acidic components can seep into the soil and hurt vegetation (1). Moreover, the acidic components of this rain can remove aluminum and calcium from the roots of plants, which can prevent them from absorbing water, with the potential to cause premature death in these plants (1). Their impact is just as severe on animals and microbes. Many animals, such as birds, are not adapted to acidic environments and thus cannot consume water from bodies of water which have a low pH level (1). Similarly, animals that are part of the aquatic food chain may die, impacting the ecosystem.

The impact to the ecosystem is taken a step further when studying microbes. Microbes, also known as microorganisms, make up a large part of the world biome. Their role is pertinent in ecosystems, nutrient cycles, and the environment (6). Microbes have a major role as decomposers, especially in fresh-water ecosystems, because they consume dead bodies of plants, animals, and other microbes (7). After consuming these organisms, they convert the decaying matter into much needed nutrients for the ecosystem, such as nitrate, phosphate, and sulfate (6). They also continue to be important in life cycles, such as the carbon and nitrogen cycles (8). Without microbial decomposers, minerals, and nutrients critical to plant and animal growth would not be made available to support other levels of the fresh-water food

chain (7).

Thus, microbes are important, especially in fresh-water ecosystems, which are key in the survival of many animals, as well as in creating products needed for the world biome to survive (9). In freshwater ecosystems, there is a unique composition of microorganisms. Fresh-water microbes span a wide range, which includes both anaerobes and aerobes (7). As well as depending on the make-up of the aquatic ecosystems, a wide variety of protists, proteobacteria, actinobacteria, Bacteroides, and cyanobacteria can be found (7). These microbes assist in performing photosynthesis and maintaining ecosystem health. The fish and other larger species that are the consumers in this food chain are directly affected by nutrient cycling and other work performed by microbes (6, 10).

The established make-up of freshwater microbes makes their response to acid rain especially important. These microbes have been shown to be clearly affected by acid rain in the natural environment, but there are few studies quantifying their response. Certain bacteria, called acidophiles, prefer a lower pH environment, but as most bacteria are not classified in this subtype, the effects of acid rain and low pH level remain unknown. A previous study found a link between lower pH and decreased biological diversity in Adirondack lakes (11). The Adirondack lakes have been forced to adapt to increased acidification, and studies like these are crucial to see how lowering pH levels in the environment may affect microbes (11). This study shows that although major microbe species did not seem to be affected significantly, there needs to be further research of the effect of pH on microbe species. Further studies tested pH levels on soil microbes and showed that lowering the pH level resulted in less microbe survival (12).

Lastly, acid rain has potential disturbances to human health (4). SO_2 can create a low ozone level, which is a pollutant damaging to the respiratory system of humans, especially young children and the elderly (4). All these consequences of acid rain can endanger all aspects of the Earth, from humans to the tiny microbes that create the base of all life on this planet.

The nature of the issue leads us to the hypothesis that agar made with a liquid that is acidic would inhibit bacterial growth compared to agar made with regular water (pH = 6.5) as shown in past studies. An acidic environment was created with vinegar; one concentration of vinegar shows current pH conditions of lakes and an increased concentration of vinegar models future pH level of lakes if we continue at the current rate. Two commonly studied bacteria, gram-negative *Escherichia coli* (*E. coli*) and gram-positive *Staphylococcus epidermidis* (*S. epidermidis*), were tested. Our results show that the data supported the initial hypothesis. It was clear that regardless of the bacterial strain, a more acidic agar environment both inhibited overall growth and decreased the rate of growth of the bacteria.

RESULTS

The dangers of acid rain were modeled in this study using the acidic agar created with a mix of water and distilled white vinegar. Different concentrations of acidic agar were created and treated with two different bacteria, gram-negative E. *coli* and gram-positive *S. epidermidis* (**Table 1**). *E. coli* is gram-negative, while *S. epidermidis* is gram-positive, which gives an opportunity to see the difference in growth based on bacterial strain. Gram-positive indicates bacteria that have peptidoglycan in the cell wall of the cell while gram-negative bacteria lack this material. Both types of bacteria are present in aquatic ecosystems and thus having two strains of bacteria with these two bacterial stains allows us to see the effects of vinegar based on bacterial stain. White vinegar is made from acetic acid, which is similar in pH to the sulfurous acid present in acid rain, and at different concentrations this helps accurately model the pH of the freshwater biome when exposed to acid rain. The different concentrations of acidic agar created a spectrum of pH levels. The regular agar solution had a pH of 6.5, agar with 6.25% vinegar had a pH of 5, agar with 12.5% vinegar had a pH of 4.5, and agar with 25% vinegar had a pH of 3.

Bacteria were left to grow for a week in an incubation container at a constant temperature of 25°C. During the week-long period, *E. coli* and *S. epidermidis* exhibited similar growth in colony count based on the concentration of vinegar (**Figures 1-2**). After the week of growth, the areas of *E. coli* and *S. epidermidis* were measured. The growth in the petri dish with no vinegar exhibited many more colonies throughout the week-long period, regardless of the strain of bacteria. For gram-negative *E. coli*, there were 6.67 times as many colonies in the agar plate with no vinegar compared to the agar plate with 25% vinegar, which exhibited only 3 colonies after 7 days (**Figure 1**). For gram-positive *S. epidermidis*, there were 10 times as many colonies in the agar plate with no vinegar compared to the agar plate with 25% vinegar, which exhibited only 1 colony after 7 days (**Figure 2**). On average, the total area (mm2) of *E. coli* colonies was much larger than that of *S. epidermidis* regardless of vinegar concentration (**Figure 3**). Similarly, the total area of growth was much larger in the agar plates with smaller vinegar concentrations

Table 1. The conditions of the different plates that were grown during this study to model the different effects of acid rain on the two bacteria used.

Variables	Group 1	Group 2	Group 3	Group 4	Group 5
Type of Bacteria	**No bacteria (Control)**	**G- E.Coli**	**G- E.Coli**	**G- E.Coli**	**G- E. Coli**
Concentration of solution mixed with agar	Regular Water	Regular water	25% Vinegar with 75% Water	12.5% Vinegar with 87.5% Water	6.25% Vinegar with 93.75% Water
Variables	Group 1	Group 6	Group 7	Group 8	Group 9
Type of Bacteria	**No bacteria (Control)**	**G+ Staph epidermidis**	**G+ Staph epidermidis**	**G+ Staph epidermidis**	**G+ Staph epidermidis**
Concentration of solution mixed with agar	Regular Water	Regular water	25% Vinegar with 75% Water	12.5% Vinegar with 87.5% Water	6.25% Vinegar with 93.75% Water

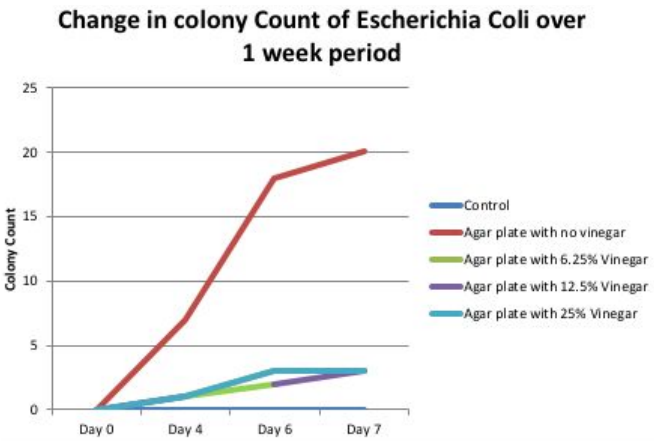

Figure 1 The growth in the number of gram-negative *E. coli* colonies over a one-week period. Each line represents a different concentration of vinegar in the agar in which the bacteria was grown. (Red: 0% Vinegar, Green: 6.25% Vinegar, Purple: 12.5% Vinegar, Light Blue: 25% Vinegar) The rate of colony count in the petri dish with 6.25% vinegar concentration is identical to the rate of growth of the 12.5% concentration, shown by the overlapping lines.

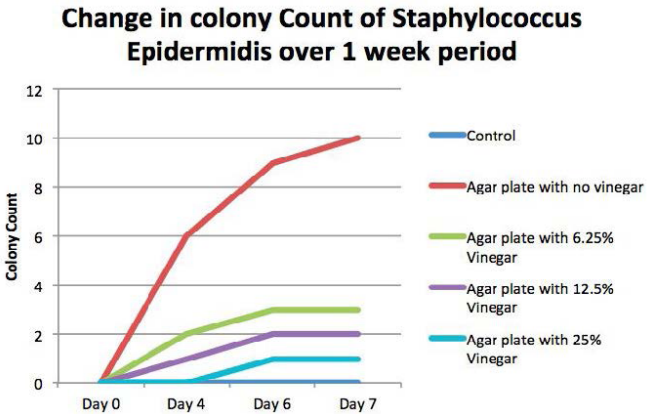

Figure 2. The growth in the number of gram-positive *S. epidermidis* colonies over a one-week period. (Red: 0% Vinegar, Green: 6.25% Vinegar, Purple: 12.5% Vinegar, Light Blue: 25% Vinegar) Growth in the petri dish with 25% vinegar concentration doesn't start until after day 4. Growth in 6.25% vinegar, 12.5% vinegar, and 25% Vinegar doesn't change from day 6 to day 7 unlike in the petri dish with no vinegar.

(**Figure 3**). For *E. coli*, there was an 89% decrease in total area from the agar with no vinegar to the agar with 25% vinegar (**Figure 3**). For *S. epidermidis*, there was a 99% decrease in total area from the agar with no vinegar to the agar with 25% vinegar (**Figure 3**). This decrease showed the clear effect that large vinegar concentrations have on bacterial growth. There was also a 48.4% decrease in *E. coli* and a 63.7% decrease in *S. epidermidis* from the agar with 6.25% vinegar to the agar with 25% vinegar (**Figure 3**). This data revealed how the pH values, which attempted to model current freshwater ecosystem levels, play a role in the decrease of bacterial growth, showing the severe effects as the concentration of vinegar increases. Individual colony size was examined and compared using a logarithmic scale with maximum, minimum, and mode intervals (**Figure 4**). Looking at *E. coli*, there was much overlap between the individual colony data, showing that colony size on average was similar in these gram-negative bacteria across different vinegar concentrations. However, in *S. epidermidis*, the data exhibited less overlap, indicating a difference in the individual size of the colonies in these gram-positive bacteria across the different vinegar concentrations. This indicated that vinegar concentration may not necessarily interfere with the size of individual bacterial colonies (**Figure 4**), as the average size of the colonies did not change significantly between concentrations, but it influenced the general number of bacteria colony growth.

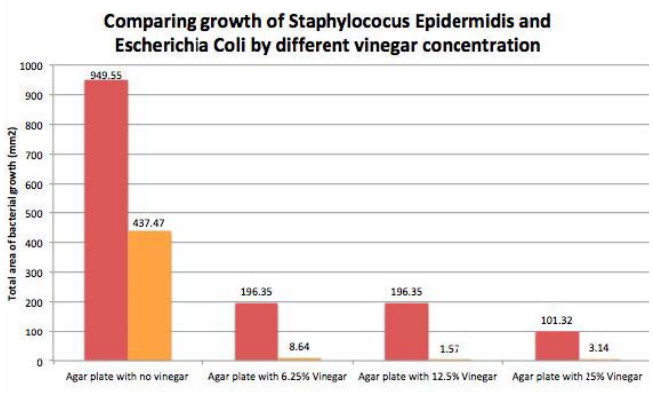

Figure 3. Comparing total bacteria growth of *E. coli* and *S. epidermidis* side-by-side based on plating conditions. (Red: *E. coli*, Orange: *S. epidermidis*) Vinegar concentration has a negative growth effect on both gram-positive and gram-negative bacteria however vinegar inhibits more growth in gram-negative bacteria.

DISCUSSION

To better understand the effect of acid rain, a two-part study was conducted to understand how acidic deposits caused by acid rain affected two bacterial strains. In this study, gram-negative *E. coli* and gram-positive *S. epidermidis* were grown in acidic agar conditions to model the effects of acid rain on the growth of bacteria.

Tests on both bacteria showed that the increase in vinegar drastically hurt the number of colonies as well as the total bacterial growth of all the colonies. The gram-negative and gram-positive bacteria grew slower in the agar prepared with vinegar versus the agar prepared without vinegar (**Figure 4**). In gram-negative *E. coli*, the bacteria grew larger with declining vinegar concentration in the agar. The total area of growth in both gram-negative and gram-positive bacteria was significantly larger in the agar plate with no vinegar (**Figure 3**). The data supported the initial hypothesis, as an increase in acid in an environment appeared to hurt the growth of bacteria in that environment. These results indicate that bacteria that are the foundation of aquatic food chains would suffer in the presence of acid rain, which could lead to a non-stable food chain in areas with higher acid concentrations.

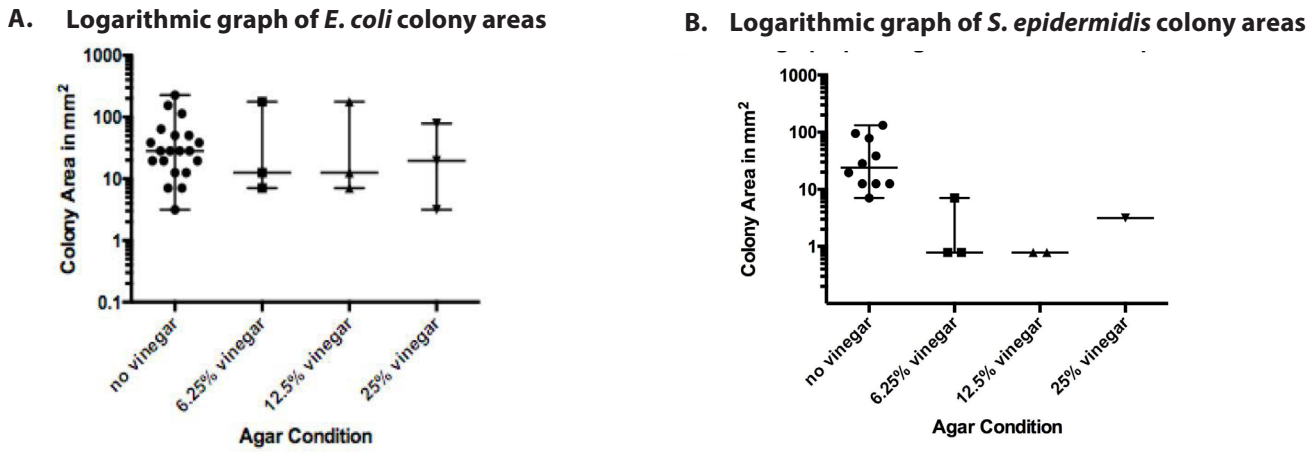

Figure 4. Logarithmic graph of individual colonies divided by vinegar concentrations. A: Individual colonies of *E. coli*. **B:** Individual colonies of *S. epidermidis*. Vinegar concentration does not appear to affect size of individual bacteria colonies but does appear to affect the number of colonies in both *E. coli* and *S. epidermidis*. Colonies were counted and measured after one week of incubated growth. Colony area is in mm^2.

This was observed via the growth curves that show the difference in growth between acidic agar and agar with no vinegar (**Figures 1-2**).

Acidic agar had notably fewer colonies compared to the control plates. This showed that the agar with vinegar is inhibiting the growth of the bacterial colonies, showing the potential destabilization of the food chain in acidic environments. An unstable food chain could lead to the elimination of many plants and animals. As well, the inhibition of growth by decreasing pH may affect nutrient cycles, as microbes play a role in the carbon, nitrogen, and phosphorus cycles, which help with photosynthesis, production of macromolecules, and productivity of the food web, respectively (6). Thus, the decrease in pH levels caused by acid rain can raise major concerns for ecosystems across the world. The pH levels of the different agar conditions accurately modeled live stream pH levels (3). The 6.25% vinegar concentration shows the pH of current streams after precipitation of acid rain. Similarly, the pH of 25% vinegar is what is expected in upcoming years if active effort is not taken to lower acid rain and subsequently increase the pH of freshwater streams.

We additionally showed that acidic vinegar affected both gram-positive and gram-negative bacteria. *E. coli* and *S. epidermidis* both showed <3 total colonies in the agar with 12.5% vinegar and even less in the agar with 25% vinegar (**Figure 4**). As the pH of agar with 6.25% vinegar models the pH of current acid rain deposits, the significant decrease in total area of bacterial growth between agar with 6.25% vinegar and 0% vinegar supports the fact that acidic conditions could be detrimental to bacterial survival (**Figure 3**). If a pH as low as 3 is reached in freshwater ecosystems, this may pose a threat to the survival of these crucial microbes, as both colony count and total area of growth is significantly decreased at this pH level.

Bacterial growth was represented by either colony count or colony size. Bacteria were equally distributed among all the petri dishes, thus colony count indicated sole areas where bacteria were able to sustain and grow. As colony count decreased with increased vinegar concentration, it seems to reveal that lower pH levels severely decrease the ability of bacteria to grow (**Figures 1-2**). Colony size, on the other hand, focuses on bacteria's growth rate and how much the bacteria can reproduce in the 7-day growth period. Colony size appeared to remain similar regardless of vinegar concentration (**Figure 4**) in *E. coli* colonies. However there appears to be a slight difference in colony size based on vinegar concentration in petri dishes with *S. epidermidis.*

It is also possible that bacterial growth could have been inhibited by factors other than the vinegar. To measure daily growth, the bacteria needed to be removed from the incubation container, and this may have caused disturbances in the growth. Possible unequal swabbing between the different petri dishes could have given disproportional initial bacteria, leading to diverse differences in the results. *E. coli* and *S. epidermidis* only account for two strains of bacteria that make up the large, diverse microorganisms on this planet and even though *E. coli* and *S. epidermidis* are not commonly found in the freshwater ecosystem, they are considered model organisms which allow them to shed light on how both bacterial strains perform under different pH conditions. Thus, these bacteria give us prime examples to model aquatic microorganisms, however it is possible that other bacteria may respond differently and that other strains may have adapted to the acidic water.

E. coli and *S. epidermis* represent all prokaryotes that may make up an ecosystem, not just a few. They are both prototrophic microorganisms, meaning they do not require a special medium with supplements to grow as they are able to synthesize their own organic material. In addition, *E. coli* and *S. epidermis* are also classified as autotrophic and heterotrophic respectively (14-15). Autotrophs and

heterotrophs are crucial in both decomposition and the nutrient cycles in freshwater ecosystems. Freshwater systems contain both types of prokaryotes (16). Though *E. coli* and *S. epidermis* are not commonly found in freshwater ecosystem, they are considered model organisms which allow them to shed light on how both bacterial strains perform under different pH conditions.

Results again provide great hope into truly understanding the depths of how microbes are affected by both pH and acid rain. This first study provides a basis for further research where bacteria and acid that more closely model nature can be tested and compared to this study in which model organisms were used. Further research can help verify the conclusions revealed in this study. To further support our conclusions, multiple data samples should be tested, and these tests should be repeated to ensure that the data is scientifically accurate. The data presented in this study revealed that we still need to address the problem facing microorganisms in aquatic environments. To model current findings, we can refer to the results from agar with 6.25% vinegar. If the world does not change and aim to reduce acid rain levels, as well as pH levels, the severe decrease in bacterial colonies and growth shown by the agar with 25% vinegar will be our new normal, causing many issues to the global environment.

METHODS

The bacteria were grown in different conditions to model the effects of acid rain on microorganism growth. To collect the data, different agar solutions were prepared with varying pH levels. Nine different groups were established with one petri dish per group. The entire experiment was performed in a Biosafety Level 1 laboratory. Dehydrated media nutrient agar was ordered via Carolina Biological Company.

Normal agar was made of 1.6 g of agar powder dissolved in 75 mL of water by boiling. Three other conditions were created to model the continual increase in acid rain in aquatic environments. Acidic solutions were modeled using a white distilled vinegar solution. The distilled vinegar had a pH of 2.4 and needed to be diluted to model pH levels of aquatic ecosystems in the world.

Current freshwater pH conditions were mimicked with a 6.25% concentration of vinegar (4.6875 mL of vinegar in 70.3 mL of water); this solution had a pH of 5. To model near future water conditions, with increasing acid rain occurrence, 12.5% vinegar (9.375 mL of vinegar) was mixed with 65.625 mL of water; this solution had a pH of 4.5. Lastly, to model potential conditions in the next decades, 25% vinegar (18.75 mL of vinegar) was mixed with 56.25 mL of water; this solution had a pH of 3. pH was measured using Litmus paper and cross-checked with a standard lab colorimeter. After measuring pH, these solutions were mixed with the dissolved agar in standard glass beakers and brought to a boil in a microwave. Each of these three acidic agar solutions were split evenly into two petri dishes creating a total of six petri dishes with acidic conditions and nine totals, including the three with standard agar.

Two different bacteria strains, *E. coli* and *S. epidermidis*, were tested. *E. coli* and *S. epidermidis* are the most accessible microorganisms available to test, as they qualify under Biosafety Level 1.

The bacteria were ordered dehydrated through Carolina Biological Company (*Staphylococcus epidermidis*- Item # 155556A, *Escherichia coli*- Item # 155065A). While the agar solidified, bacteria were rehydrated using a provided medium and incubated at room temperature for 30 minutes. When the agar was at the right solid consistency, sterile inoculating loops were dipped in the respective bacteria and used to apply an even coating of each bacteria on the given petri dish. The use of the inoculating loop with the same number of bacteria was used to ensure even distribution of bacteria in each petri dish, preventing another defining variable. Inoculating loops were placed in a biohazard bag after one-time use. One petri dish was swabbed with an unexposed inoculating loop to establish a control.

After dish preparation, nine petri dishes with nine unique conditions were created to measure. The variables for this project were the concentration of vinegar in the agar solution and the bacterial strain of the bacteria. All nine petri dishes were sealed with tape and labeled appropriately. After this, they were placed in an incubation container and left at 25°C for seven days. Bacteria were measured on four days: day 0, day 4, day 6, and day 7, and the number of colonies were counted, and their growth was compared from the previous day of measurement. Besides this measurement, the bacteria were kept undisturbed for the week-long growth period.

After one week of growth, the bacteria colonies were counted, the size of the colonies was measured, and then the petri dishes were appropriately disposed of. The size of the colonies as seen in **Figures 3-4** was measured by outlining each colony on the petri dish and using a ruler and protractor to estimate the size of each colony.

Figures showing data of the bacteria growth over the week as well as comparisons of gram-negative *E. coli* and gram-positive *S. epidermidis* were created with Prism 6 program. **Figure 3** represents the $\log_{10}$ size of individual bacteria colonies. The box plots represent maximum and minimum size with the middle line representing the mode size of colonies.

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REFERENCES

1. Nunez, Christina. "Acid Rain facts and information." *National Geographic*, 28 Feb. 2019 https://www.nationalgeographic.com/environment/global-warming/acid-rain/.
2. Madaan, Sonia. "Primary Causes of Acid Rain." *Earth Eclipse*, 17 Apr. 2016, www.eartheclipse.com/environment/primary-causes-of-acid-rain.html.
3. "What Is Acid Rain?" *EPA*, Environmental Protection Agency, 18 Apr. 2019, www.epa.gov/acidrain/what-acid-rain.
4. Boumis, Robert. "Negative Health Effects of Acid Rain on Humans." *Sciencing*, 19 June 2019, https://sciencing.com/negative-health-effects-acid-rain-humans-24007.html.
5. Abrahamsen, G., Hovland, J., and Hagvar, S., Effects of artificial acid rain and liming on soil organisms and the decomposition of organic matter, in: Effects of Acid Precipitation on Terrestrial Ecosystems, pp. 341–362. Eds T. C. Hutchinson and M. Havas. Plenum Press, New York 1980.
6. *Acid Rain*. http://nadp.slh.wisc.edu/educ/acidrain.aspx. Accessed 7 Mar. 2020.
7. Chapter 5 ~ Flows and Cycles of Nutrients. (2020, August 21). Retrieved August 21, 2020, from https://ecampusontario.pressbooks.pub/environmentalscience/chapter/chapter-5-flows-and-cycles-of-nutrients/.
8. Robinson, R., & Clark, N. (n.d.). Microbes in Lakes and Streams - river, important, system, plants, source, oxygen, human. Retrieved August 20, 2020, from http://www.waterencyclopedia.com/La-Mi/Microbes-in-Lakes-and-Streams.html#:~:text=Leaves%20and%20branches%20that%20fall,available%20for%20other%20aquatic%20creatures.
9. UNC Institute for the Environment. (n.d.). Investigating Freshwater Microbes and Their Role in the Carbon Cycle. Retrieved August 20, 2020, from https://ie.unc.edu/files/2014/12/Freshwater-microbes-lessons-FINAL.pdf.
10. Dodds, W., & Whiles, M. (n.d.). Freshwater Ecosystem - an overview | ScienceDirect Topics. Retrieved August 21, 2020, from https://www.sciencedirect.com/topics/earth-and-planetary-sciences/freshwater-ecosystem.
11. Society, N. (2019, August 2). Freshwater Ecosystem. Retrieved August 21, 2020, from http://www.nationalgeographic.org/article/freshwater-ecosystem/.
12. Rensselaer Polytechnic Institute (RPI)Database Shows Effects of Acid Rain on Microorganisms in Adirondack Lakes. (n.d.). Retrieved August 20, 2020, from https://news.rpi.edu/luwakkey/2461.
13. Xu HQ, Zhang JE, Ouyang Y, *et al*. Effects of simulated acid rain on microbial characteristics in a lateritic red soil. *Environ Sci Pollut Res Int*. 2015;22(22):18260-18266. doi:10.1007/s11356-015-5066-6.
14. Blount Z. D. (2015). The unexhausted potential of E. coli. *eLife*, *4*, e05826. https://doi.org/10.7554/eLife.05826.
15. Animals of Freshwater. (2015, April 5). Retrieved September 9, 2020, from https://askabiologist.asu.edu/animals-freshwater.
16. Lab-Evolved E. coli Consume Carbon Dioxide. (n.d.). Retrieved September 9, 2020, from https://www.the-scientist.com/news-opinion/lab-evolved-e--coli-makes-energy-solely-from-carbon-dioxide-66788.
17. Staphylococcus Aureus: Structure and Function. (n.d.). Retrieved September 9, 2020, from https://www.ukessays.com/essays/biology/identification-of-staphylococcus-aureus-from-bacterial-genus-biology-essay.php.
18. "How to Grow Bacteria: Lesson, Science Projects, Video." *Home Science Tools*, 12 Feb. 2019, learning-center.homesciencetools.com/article/bacteria-experiment-guide/.3.
19. Francis, A.J. Acid rain effects on soil and aquatic microbial processes. *Experientia* 42, 455–465 (1986). https://doi.org/10.1007/BF01946683.
20. Liang, X., Liao, C., Thompson, M. L., Soupir, M. L., Jarboe, L. R., & Dixon, P. M. (2016). *E. coli* Surface Properties Differ between Stream Water and Sediment Environments. *Frontiers in microbiology*, *7*, 1732. https://doi.org/10.3389/fmicb.2016.01732.
21. Pandey, P. (2012). *Modeling In- Stream Escherichia coli Concentrations*. Iowa State University. Retrieved from https://lib.dr.iastate.edu/cgi/viewcontent.cgi?referer=https://www.google.com/&httpsredir=1&article=3862&context=etd.