Does Sunlight Affect Bacteria Density?

By: Rachael Barnes, Valerie Bandell, Rebecca McWilliams, Bethani Williams

Abstract:

Bacteria are an essential component of all ecosystems. Following the E.S.S.R.E 2005 biota survey, we discovered that the bacteria levels in E.S.S.R.E. site four were unusually low. Previous research in E.S.S.R.E. site four shows that the protozoa and mold densities are affected by sunlight. We decided to test if that was true for bacteria as well. We selected 3 research plots in sunny areas of site four and divided each plot into 5, 20cm x 20cm squares. We took 3 soil samples from each square, with a 2cm soil core, before we covered them with mesh. We covered the squares with varying numbers of mesh pieces to manipulate the amount of soil received by the soil. We took 3 soil samples after the squares had been covered with mesh for 5 days. We conducted serial dilutions on all the soil samples and plated them on 3M Petrifilm® plates. We counted the bacteria on the plates after a couple days and estimated the density of bacteria in the original soil sample. We found the percent of change in density of bacteria before and after the experiment. The results of our experiment showed that the large amount of sunlight did not have an effect on the bacteria and so we concluded that something other than the sunlight is causing the bacteria population to decrease. We believe that further research concerning the relationship between bacteria and water is needed. To do this we would manipulate the amount of water received by the soil instead of the amount of sunlight.

Introduction:

The ecosystem depends on the function and role of the bacteria in the soil. The bacteria are in charge of cycling chemicals and nutrients such as carbon and nitrogen. The cycling of these nutrients, especially nitrogen is important to the plant life which depends on the nitrogen in the soil to remain healthy and continue growth. The plants take the nitrites and nitrates through their roots and use them to make proteins which help them grow. The bacteria take in the nitrogen from the air, during a process known as nitrogen fixation, and convert it to ammonia. Then, other bacteria further convert the ammonia into nitrites or nitrates which the plants then use for nutrients (CHM 110- Chemistry and Issues in the Environment, "n.d."). Some bacteria however do the reverse and take the nitrites and nitrates from the soil and convert those nutrients to the gaseous nitrogen in the air. The heterotrophic bacteria also break down dead organisms in the environment providing organic compounds which are used for energy. The bacteria are then able to return nutrients to the ecosystem. The low population of bacteria affects the density of the plants. Without a sufficient amount of bacteria the soil lacks the nutrients needed by the plants and will eventually cause them to die (Speer, Waggoner, 1995).

Plant diversity and microbe population have been decreasing at the E.S.S.R.E. research site four (E.S.S.R.E. 2001-2005). Previous research groups at E.S.S.R.E. found that ultra violet rays have decreased the density of mold and protozoa in site four (Bartlett, Edison, Sorace, 2002; Khandelwal, Murphy, Ravi,

2004). While researching in site four this year (North 39.35772, West 076.6389) we also noticed the low numbers of mold and protozoa as well as bacteria (E.S.S.R.E. 2005). Since bacteria and mold need the same conditions for growth, it would make sense that if there are low levels of mold, there will also be low levels of bacteria (Kansas State University 2005: Wall 1999). We know that bacteria in site four have been decreasing from previous years (E.S.S.R.E. 2001-2005).

Normally, site four receives a lot of sunlight and is well watered. There is an abundance of jewel weed but few trees and other plant diversity. There is a stream that runs throughout the site and is a marsh like surrounding. The soil is very silty with small amounts of clay and sand (E.S.S.R.E. 2005). Based on the geography of site four, we would expect the mold, bacteria, and protozoa levels to be higher because the site has more ideal conditions for the growth of these microbes. Mold thrives in most damp, warm, and humid places. Mold also grows rapidly in dark places. Under these conditions the mold levels should be high (Kansas State University 1995). Protozoa use the organic compounds released by the bacteria's decomposing of dead organisms for energy and nutrition. They also like damp areas and warm temperatures (Stevenson 2004). Bacteria also have these same basic needs of water, humidity, darkness and warm places to flourish in an environment (Wall 1999). Site four, with the streams and the warm temperatures, would be an ideal place for the mold and bacteria to thrive. However, site four cannot provide the darkness needed for the mold to obtain a

high population level and the ultraviolet rays are decreasing the density of protozoa in the environment (Khandelwal, Murphy, Ravi, 2004). The other sites differ greatly in description and location from site four and provide the darkness needed for the mold and protozoa to prosper. Site three (N 39.35833; W 076.6386) is on a hilltop and very steep and sloped. The source of the stream is located here. It is heavily populated with rhododendrons and English ivy. The soil is mostly sand but does contain about a quarter silt and a quarter clay. Site two (N 39.35837; W 76.63892) is an eroded stream bed lined with deciduous plants and heavy undergrowth. The stream runs along the remnants of a concrete dam (E.S.S.R.E. 2004). In these sites the mold and protozoa levels were higher because of the different physical environment.

In areas where there is not a lot of sunlight, bacteria thrive and maintain a good population. In the presence of sunlight the bacteria become inactive and eventually die. Visual light may be the cause rather than the ultra violet for the effect and decrease in the bacteria population (Fujioka, Hashimoto, Siwak, Young 1981). When diluted in water the bacteria become even more inactive. The sunlight stresses the bacteria resulting in cell death. (Fujioka, Narikawa, 1982). Based on this information, we are experimenting to see if sunlight is what is effecting the bacteria population in site four.

Methods and Materials:

We took 90 soil samples from 3 different research plots within 20 meters of one another between July 19 and July 25, 2005. The research plots are located in

open spaces in E.S.S.R.E. Site 4 on the Roland Park Country School campus. Site 4(N 39.35772,W 076.6389) is a wetland meadow that has developed from the filling in of the old farm pond. The stream flows through the middle of Site 4 as it moves down the hillside, and there are almost no trees and little plant diversity. A very large area of jewelweed covers one corner of this research site.

The first research plot we took samples from is very open. There is very little ground covering; only some sparse weeds. The soil is very damp. It is located about 1.5 m from a small stream. The plot is overlooked by slender tree branches and the sunlight easily shines through. This plot receives the second most sunlight out of the three plots. The second research plot is located on the inner edge of the jewelweed patch. The soil here has more moisture than the soil in research plot 1. It is about 1 meter from the other side of the stream. It receives the most sunlight out of all the research plots. The third research plot is located on the outer edge of the jewelweed patch. It is on the side opposite research plot 2. The soil here is very sandy and contains the least moisture out of all three research plots. It lies in what was formerly a stream bed. This plot is on the same side of the stream as plot 2, however it is further south. It receives the least amount of sunlight out of all three research plots. All three research plots are located in the same wetland meadow.

Stage 1:

Each research plot consisted of 5, 20cm x 20cm squares lined up next to each other. We placed flags on the corners of each square in order to identify them. We took three soil samples from the 1st square in each plot but we did not

cover it with mesh. We used a 2mm soil core to get the soil samples. We pushed the soil core 15 cm into the ground and then twisted the soil around at least 360°. We then pulled the soil core out of the ground and emptied the collected soil into a small plastic bag. The amount of soil normally reached the 15 cm mark on the soil core. The soil core must be rinsed out between each sample. We took soil samples for the second square in each plot using the same method. After we had taken three soil samples from the second square, we placed one 20cm x 20cm square of 1 mm black nylon mesh on top of it. We placed the flags through the corners of the mesh to hold it in place. After we took 3 soil samples from the second square in each plot, i we placed 2 pieces of mesh over the selected area not 1.On the fourth square in each plot we placed 3 pieces of mesh on top of the square area. We placed 4 squares of mesh on top of the fifth square in each plot after we had taken three soil samples from it.

Serial Dilutions:

After we took all the soil samples from the first stage, we conducted serial dilutions for the all the soil samples. We did all the soil samples from one site simultaneously. We diluted them to 10^{-4}. When we had completed all the dilutions of the samples, we plated 100µl of them on 3M Petrifilm® plates. We allowed them to grow for 5 days.

Stage 2:

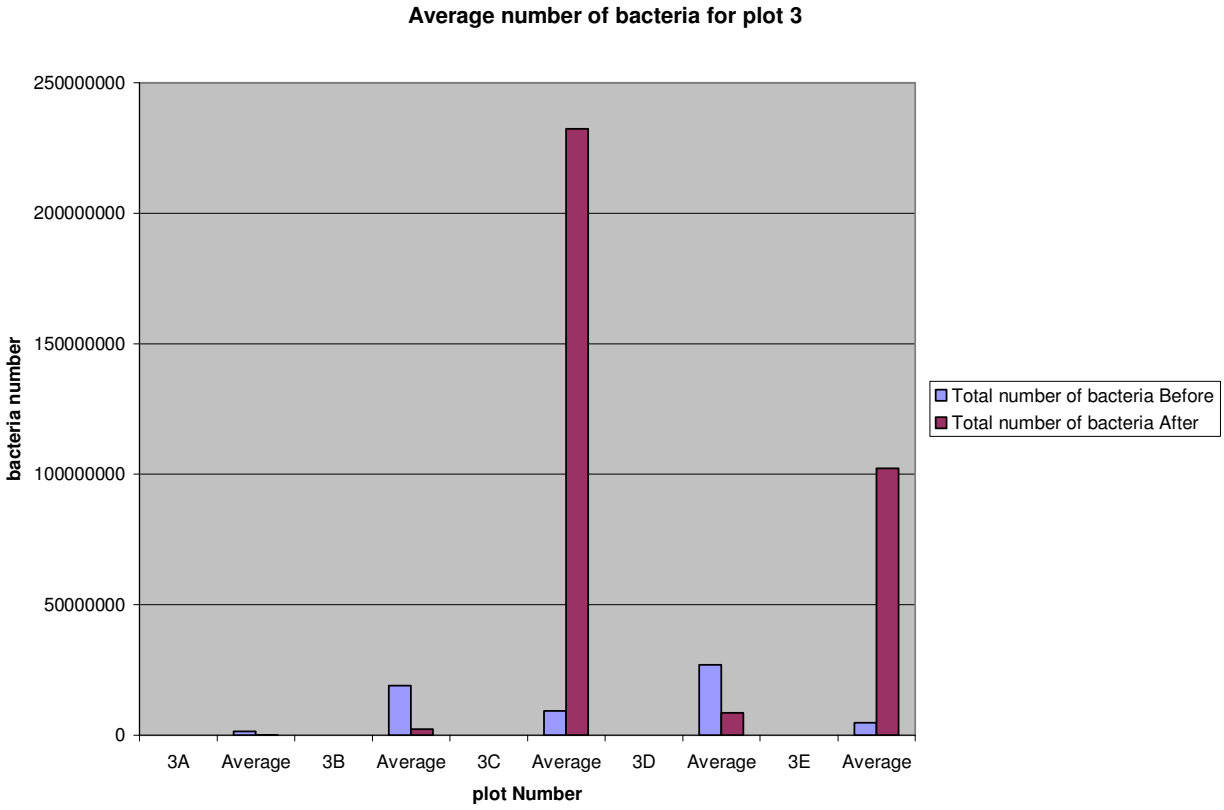
We allowed the sites to remain undisturbed for 5 days. On the 6^{th} day after the original soil samples were taken, we went back out to the research plots and

took three more soil samples from each square in each site. Once all 45 samples from stage 2 had been obtained, we performed serial dilutions on them. We also diluted these samples to 10^{-4}. When the serial dilutions for the soil samples were completed, we plated 100µl of all of the samples on 3M Petrifilm® plates. We allowed the Petrifilm to sit in a cool dark place for 3 days. We then counted the number of colonies on the most dilute sample in order to estimate the number of bacteria in the original soil sample. We computed the percent of change in the density of bacteria before and after the experiment for each condition.

Results:

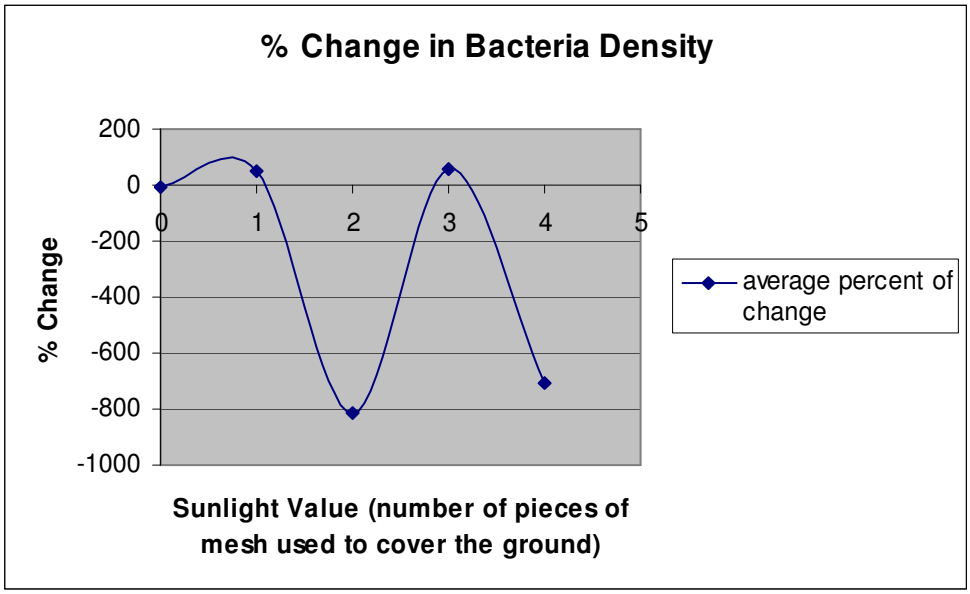
During our analysis, we averaged the three numbers of the counted bacteria from each square. We did this for every research plot and generated bar graphs (see graph 1) to observe the pattern between density levels before and after the experiment.

Graph 1:



We realized we needed to calculate the percent of change in bacteria density because this graph shows no recognizable pattern.

Graph 2:



Graph 2 shows the percent change in the amount of bacteria in relation to the amount of sunlight. Clearly, there is no recognizable relation between the amount of sunlight and the density of the bacteria. Because of that, we wondered if our experiment actually changed the environment. The pattern shown on the graph was erratic and so we t-tested the individual numbers from each condition against the numbers of the control condition. We did this for the bacteria numbers we had estimated from stage one and the bacteria numbers we had estimated from stage two.

Chart 1:

Before Sunlight Manipulating	plot number	P-Values	After Sunlight Manipulating	plot number	P-Values
negative control 0 (A)	vs. B	.06	negative control 0 (A)	vs. B	.209
negative control 0 (A)	vs. C	.02	negative control 0 (A)	vs. C	.224
negative control 0 (A)	vs. D	.03	negative control 0 (A)	vs. D	.189
negative control 0 (A)	vs. E	.04	negative control 0 (A)	vs. E	.302

Chart 1 shows the results of the t-testing of the data from each of the sites. Clearly, the p-values show that there were significant differences in the populations of bacteria before the experiment started. However, the p-values following the experiment show that there was no longer a significant difference in the populations of bacteria. Therefore, we concluded that the experimental process did change the environment in which the bacteria were living somehow.

Discussion:

Our results clearly contradicted our hypothesis. Our hypothesis stated that the amount of sunlight affected the growth of bacteria and it caused the bacteria to decrease in numbers. We thought that a decrease in the amount of sunlight would cause an increase in the density of the bacteria. However, the results from graph 1 showed that the amount of sunlight was not affecting the density of bacteria. According to our hypothesis, the graph should have shown an increase in the density of the bacteria as the amount of sunlight able to reach the soil decreased.

In reality the graph showed that decreasing degrees of sunlight had no permanent pattern in relation to an increase of bacteria.

This was very surprising since site four receives a lot of sunlight, and we learned that bacteria cells can die from the sunlight (Wall 1999). The outcome of graph 1 showed us that we were obviously incorrect about the cause of the decreasing bacteria population.

We then began wondering why our hypothesis was wrong. As we stated in our introduction, if there was a lower density of mold, then there would also be a decreasing density of the bacteria (Kansas State University 2005: Wall 1999). After we found a large amount of information about the sunlight and Ultra Violet Rays decreasing large amounts of protozoa life in Site 4, we thought for sure that this was the cause for the decreasing density of bacteria (Khandelwal, Murphy, Ravi, 2004).

We decided to t-test the bacteria samples to see if there were significant differences between the densities of bacteria in the soil prior to when we did the experiment. By comparing the density of the bacteria in each of the four independent variable conditions to the negative control condition before actually putting the mesh down, we noticed that the P values were significantly different (See Chart 1). But when we t-tested the density of the bacteria in each of the four independent variable conditions to the negative control condition a second time to find the P values, we were surprised to find out that all of the results were almost identical. The results were supposed to be significantly different just as the results

from the first bacteria count were (see chart 1). We realized that while the variation in the amount of mesh that we used did not alter the bacteria density (see graph 2), something about our experiment changed what was actually happening to the bacteria densities.

Because our hypothesis was wrong, something other than the sun was causing the bacteria to die. It is possible that, because this type of bacteria is heterotrophic and does not make its own food, the bacteria could be dying because whatever they eat could be decreasing as well (Speer and Waggoner 1995).

Another possible cause could be that the bacteria became diluted in the rain water (E.S.S.R.E 2005) the day that we collected the "after" samples. Rain can cause bacteria to become inactive and eventually die (Fujioka, Narikawa, 1982). In order to discover if the excessive rainfall caused the decrease in the density of the bacteria, further research can be done concerning effect of water on bacteria. The amount of rain water would be the control in an experiment concerning this idea.

Acknowledgements:

We would like to thank everyone for making this summer a possibility and providing all of us with this experience to learn and expand our science education. The first people we would like to thank the Waksman Foundation for Microbiology for our funding this year. We would also like to thank Mr. David Brock, our mentor and teacher throughout the program. With his guidance and

knowledge we learned about soil and microbiology and were able to complete our research experiments, papers, and websites.

Bibliography:

Bartlett, E.; Edison, P.; Sorace, C. (2002) "The Effects of Ultraviolet Radiation of the Mold Population Density in Soil". *E.S.S.R.E. 2002* Available online: http://faculty.rpcs.org/brockda/ESSRE%202002%20Student%20Webs/ESSRE%202002%20(uv%20+%20mold)/UV%20Research.pdf

Bode, M. (1995). *Controlled Mold Growth in the Home* Available online http://aginfo.psu.edu/News/july99/bacteria.html

Elmhurst College, (n.d.). *Nitrogen Cycle* Available online http://www.elmhurst.edu/~chm/onlcourse/chm110/outlines/nitrogencycle.html

E.S.S.R.E. (2005) "2005 Site 4 Summary Information" *Environmental Science Summer Research Experience for Young Women* Available online https://faculty.rpcs.org/brockda/Survey%20Data/2005%20Site%204%20Summary.htm

E.S.S.R.E (2001-2005). "Research Summary" *Environmental Science Summer Research Experience for Young Women* Available online https://faculty.rpcs.org/brockda/Survey%20Data/ESSRE%20Data%2001%20to%2005.htm

Fujioka, R. (1982). "Effect of Sunlight on Enumeration of Indicator Bacteria Under Field Conditions", *Applied and Environmental Microbiology*, 44(2), 395-401.

Fujioka, R. S., Hashimoto H. H., Siwak E. B., and Young, R. H. (1981). "Effect of Sunlight on Survival of Indicator Bacteria in Seawater", *Applied and Environmental Microbiology*, 41(3), 690-696.

Penn State(1999). *SUMMER HEAT AND HUMIDITY PROVIDE BACTERIA-FRIENDLY CONDITIONS THAT BACKYARD GRILLERS SHOULD BE AWARE OF* Available online http://aginfo.psu.edu/News/july99/bacteria.html

Speer, B.R; and Waggoner B. (1995) “Bacteria: Life History and Ecology” *Museum of Paleontology, University of CA, Berkley*. Available online http://www.ucmp.berkley.edu/bacteria/bacterialh.html