Emory Gatchell, Oyinade Oyenusi, Lilly Ratcliffe, Ajée Robinson

Mr. Brock

Biology 9H

27 April 2016

Pesticides and Protozoa

By: Emory Gatchell, Oyinade Oyenusi, Lilly Ratcliffe, and Ajée Robinson

Emory Gatchell, Oyinade Oyenusi, Lilly Ratcliffe, Ajée Robinson

Mr. Brock

Biology 9H

27 April 2016

Background

Protozoa are a group of single celled eukaryotic organisms that are classified based on how they move in order to ingest food, and in the soil, there are three main types: ciliates, amoebae, and flagellates. Ciliates, the largest in size, move by means of hair like projections, and while they eat mainly bacteria just like the other two types of protozoa do, they also eat the other two types of protozoa. The second kind of protozoa, the amoebae, travel by "foot" in order to capture their prey (meaning they move by expanding and contracting their cell membrane), and finally, the flagellates, the smallest of the protozoa, use whip-like flagella or “tails” to move, in order to find bacteria to eat (Ingham n.d.). All three groups play key roles in the soil ecosystems, and one of the most important of these is the critical part protozoa play in the soil food web.

Because protozoa control the amount of bacteria in the soil by consuming them, after eating the bacteria, the protozoa transfer their energy up the food chain to larger organisms, such as flatworms and nematodes. Together, the protozoa, bacteria, and worms maintain a stable cycle of organic matter in the soil, contributing to the overall health of the soil, and one especially important material they move is the element nitrogen. Soil bacteria contain levels of nitrogen that exceed the ratio of carbon to nitrogen needed by a protozoa to survive; therefore, when protozoa eat bacteria, they release most of this extra nitrogen into the soil in the form of ammonium, allowing plants and other microbes to absorb it for their own metabolic needs. This cycling process is crucial because microbes (such as bacteria, fungi, and protozoa) and multicellular organisms (such as nematodes and arthropods) are always transforming nutrients between two forms: inorganic compounds that are dissolved in water or attached to minerals, and organic compounds that are part of living organisms and dead organic matter. When they ingest inorganic compounds and organic compounds, they are said to be immobilizing nutrients, and when they excrete inorganic waste compounds, they are said to be mineralizing the nutrients (Ingham n.d.). But when they mineralize nutrients, they are making any nitrogen in these elements useable to plants in the forms of ammonium and nitrate. While bacteria alone are responsible for turning ammonium into nitrate through the process of nitrification (National science foundation 2016), protozoa contribute to this process, due to the speed at which they consume bacteria and release the excess ammonium they do not need into the soil. This pace of consumption helps to maintain the correct population balance of bacteria and therefore the correct amount of nitrogen needed for the health of soil (Ingham n.d.).

The reason the plants and other organisms need the nitrogen provided by the bacteria and protozoa is because without nitrogen in the soil (or anywhere), no amino acids can be made, therefore enzymes cannot be constructed. Without enzymes in cells of livings things, no chemical reactions will happen. If that were to happen, then the cells would not be able to function, causing everything living in the soil to be dead. Therefore, the element nitrogen is crucial to have in usable forms in the soil so that chemical reactions are able to take place, keeping the entire ecosystem alive.

For our experiment, we are testing how the amount of protozoa in the soil could be decreased. A commonly used chemical that could potentially decrease the amount of protozoa in the soil is pesticides. Pesticides are chemical sprays that are used on plants in order to keep pests such as beetles, slugs, caterpillars and crickets from eating the plants, but when the pesticide is applied to plants, the soil where those plants live is being exposed to several dangerous chemicals. Although pesticides are only meant to kill insects that could harm the plants, they may also harm the soil by killing important microbes and other organisms that help with the health of the soil. Chemicals such as Diazinon, Fenthion, Malathion, Methyl-parathion, Sulfotep, and Trichlorfon are common pesticides, and they are classified as being organophosphates. These chemicals destroy insects by attacking their nervous system and killing them

(Gerber 2016). Specifically, organophosphates kill insects by irreversibly inactivating acetylcholinesterase, which is necessary for an insect's nervous system to function. The organophosphates essentially poisons the enzyme, causing it to break down in the insects (CDC n.d.). But in the soil, these organophosphates increase numbers of protozoa when applied to the soil because they increase the number of bacteria that are decomposing it for their own needs, providing additional food for the protozoa to eat and multiply in number (Department of Environmental Science, Lancaster University, UK.2004).

If the pesticides are applied to the soil and they cause the density of protozoa to increase, then there will be more protozoa to eat the bacteria. But this could be problematic because all this consumption will decrease the bacteria population, potentially causing issues in the nitrogen cycle, because if there are not enough bacteria to complete the ammonification and assimilation processes, then there will be a limited amount of nitrogen in the soil and there may not be enough nitrogen to make enzymes. On the other hand, if the pesticides decrease the amount of protozoa, then there will not be enough protozoa to eat all of the bacteria, so there will be an excess amount of bacteria which can lead to the bacteria overtaking other microbes.

After researching protozoa, we have decided to test if Bayer multi use pesticides increases or decreases the amount of protozoa in the soil. We plan to set up six plots (three for negative control and three for the use of pesticides) to collect soil samples before and after we apply pesticides to three of the plots, and water as our negative control to the other three plots. We will then test the soil from the plots to see how many protozoa are in each sample. After we test, we will know if the pesticides increased or decreased the amount of protozoa in the soil.

Experiment

I. Problem: Does the presence of Bayer Multi-Use Pesticide increase or decrease the density of protozoa in the soil?

II. Hypothesis: The presence of pesticides will decrease the density of protozoa in the soil.

III. Procedure:

A. Independent Variable:The application of pesticide to the soil

B. Dependent Variable: The density of protozoa in the soil

C. Negative Control: The application of only water to the soil

D. Positive Control: The samples of soil taken before applying the pesticides and water

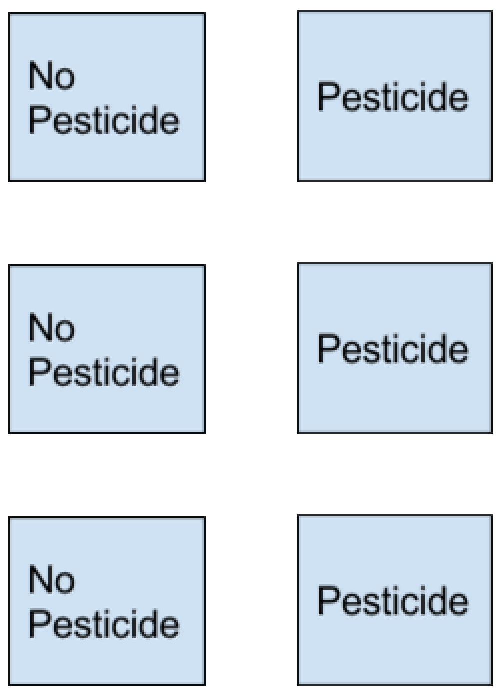
E. Controlled Variables:

1. The type of pesticide
2. The amount of pesticide sprayed on the grass
3. The amount of water sprayed on the grass
4. The location of the soil used
5. The method of application of the spray
6. The time/day the soil is collected
7. The amount of soil collected from each plot
8. The size of the plot
9. The distance between plots
10. How the flags are positioned
11. How many grams of soil are sifted into the 2nd petri dish
12. The amount of distilled water used to saturate the soil
13. How long the petri dishes are left out to rehydrate
14. Size of the filter paper/type of filter paper
15. Amount of methyl-green stain used
16. Size of the coverslip
17. Magnification of the light microscope
18. How long the pesticide sits on the soil.

19. How long the soil is left out to dry
20. How long the soil sits in the Uhlig extractor
21. The amount of water in the Uhlig extractor
22. The amount of filtrate added to the stain on the microscope slide

F. Step-by-Step Instructions:

1. Get 24 flags and position them at the following coordinates (N 39^0 21.413’ W 076^0 38.175’) in the following grid pattern:



The flags should be at each corner of the plot. Each plot should be 30cm by 30cm and have a 20cm distance on all sides to separate it from the next plot.

2. On the same day at the same time, use a soil core extractor to collect three soil samples from each of the six plots that are 15 cm deep with a diameter of 2 cm from each plot of soil. Do this before

spraying the pesticide or water on the plots. Then put soil in labeled plastic bags. These bags should be labeled with:

a. The plot number

b. Whether the sample was taken before or after the water and pesticides were sprayed

c. Whether the sample was taken from the negative control side or the pesticide side

d. The soil sample number

- For example:
  - "Plot 1, Before, Negative Control, Sample 1"

3. Once you are back at the lab station, test for the density of protozoa using the following steps:

4. On the same day at the same time, place each soil sample into the bottom of its own clean, empty petri dish and allow to dry completely. Label each petri dish with the same instructions that were used to label the plastic bags. Make sure each bag of soil goes into its corresponding petri dish.

5. Using a 1 mm^2 nylon screen or mesh, sift 9-10 grams of soil into a 2nd clean petri dish and record the exact amount for each sample. Label each petri dish with the same instructions that were used to label the zip lock bags. Make sure each bag of soil goes into its corresponding petri dish.

6. On the same day at the same time, add 20 ml of distilled water to each petri dish to saturate the soil.

7. Cover all petri dishes and allow to sit for 7 hours.

8. On the same day at the same time, place each of the resaturated soil samples in their own modified Uhlig extractor containing 30 ml of distilled water for 24 hours.

9. Remove the filtrate from each Uhlig extractor and filter a 2nd time using 12.5 cm qualitative filter paper.

10. On the same day at the same time, use a capillary tube to deposit 7 µl of methyl-green stain on a separate clean microscope slide corresponding to each sample (1 µl = 1 drop from the capillary tube).

Then using a disposable graduated Beral-type pipette, add 18 µl (the first demarcation on the pipette) of the 2nd filtrate from each sample from step 9 to the stain on each corresponding microscope slide and cover each one with an 18 x 18 mm^2 coverslip. Be sure to label the slides according to their original soil sample.

11. Examine each sample under a light microscope at 40X observations of the various protozoa living in the soil. Count the protozoa from five different fields of view and average the results.

12. Use the following equation to determine the population density of protozoa in the soil sample: [(# per field of view at 40X) • (total ml of water used) • 747] / (grams of sifted soil) = # of protozoa per gram of soil.

13. Record data in the data table and calculate the average of each trial of each soil sample.

14. On the same day at the same time, spray 10 squirts of water on each of the three negative control plots and spray 10 squirts of pesticide on each of the three pesticide plots. Allow the pesticide to sit for at least 24 hours.

15. Now that the water and pesticide has been sprayed, repeat steps 2-13.

Analysis

The Number of Protozoa Per Gram of Soil Before Sprayed:

Trials	Negative control	Pesticides
Plot 1 sample 1	180,254	115,298
Plot 1 sample 2	212,148	216,478
Plot 1 sample 3	138,729	216,712
Plot 2 sample 1	184,314	94,998

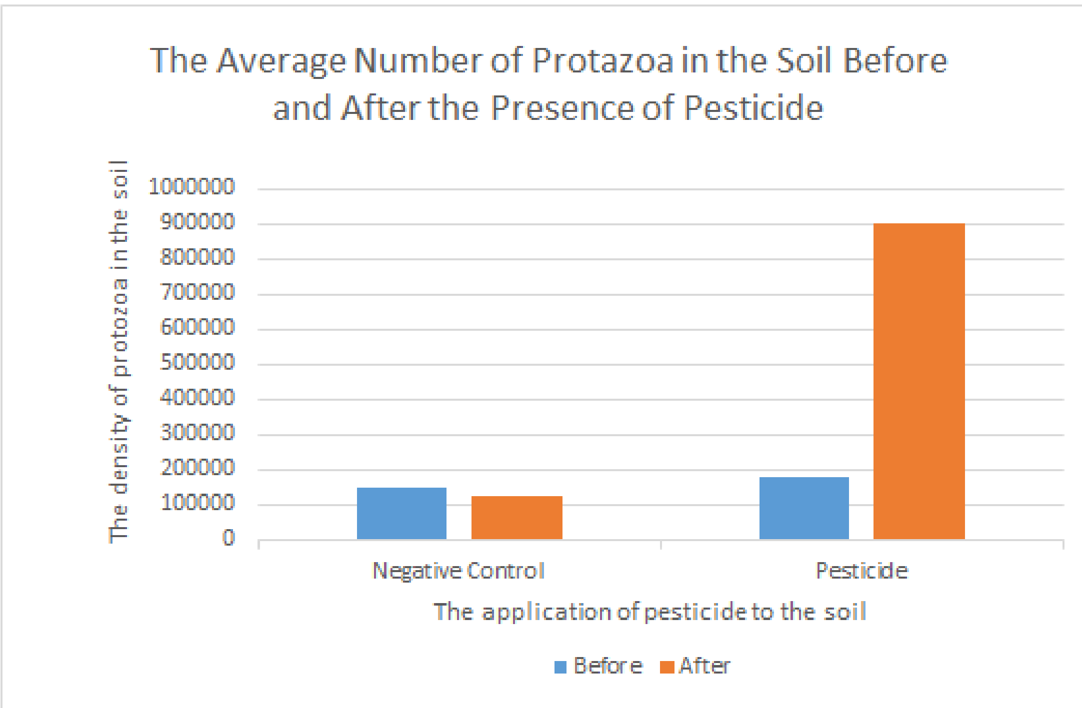
Plot 2 sample 2	234,542	26,681
Plot 2 sample 3	106,391	204,399
Plot 3 sample 1	123,132	176,194
Plot 3 sample 2	92,960	175,668
Plot 3 sample 3	57,462	150,212
Average	147,770	179,212

<u>The Number of Protozoa Per Gram of Soil after Sprayed:</u>

Trials	Negative control	Pesticides
Plot 1 sample 1	191,265	37,760
Plot 1 sample 2	135,445	432,430
Plot 1 sample 3	111,640	1,141,022
Plot 2 sample 1	82,088	307,930
Plot 2 sample 2	181,770	78,020
Plot 2 sample 3	84,443	5,212,158
Plot 3 sample 1	97,435	504,841

Plot 3 sample 2	154,736	82,481
Plot 3 sample 3	78,591	281,858
Average	124,157	897,611

Graph:



Conclusion

In conclusion, our hypothesis was incorrect. We hypothesized that the presence of pesticide in the soil would decrease the density of protozoa found. The average number of protozoa was 147,770 for the negative control before water was sprayed, 179,212 before pesticide was sprayed, 124,157 after water was sprayed, and 897,611 after pesticide was sprayed. After the water was applied to the negative control plots, the number of protozoa decreased by an average of 23,613. After the pesticide was applied to the pesticide plots, the number of protozoa increased by an average of 718,399. This goes to show that the presence of pesticides increases the amount of protozoa in the soil because with pesticides, the amount of protozoa per gram of soil increased by 718,399 whereas water decreased the amount of protozoa per gram by 23,613. As shown by our data, the application of pesticide significantly increased the number of protozoa in the soil compared to when only water was applied to the soil. Therefore, the presence of pesticides in the soil greatly increased the density of the protozoa.

We thought that the presence of pesticides would decrease the amount of protozoa, and because there would not be enough protozoa to eat all of the bacteria, there would be an excess amount of bacteria which could lead to the bacteria overtaking other microbes. We thought that the organophosphates would poison the protozoa and therefore decrease the amount of protozoa which could lead to overpopulation of bacteria, having them take over the other microbes.

But in the soil, these organophosphates increased the amount of protozoa when applied to the soil because they increased the number of bacteria that are decomposing it for their own needs, providing additional food for the protozoa to eat and multiply in number. Past studies have also shown the same patterns of the protozoa increasing when they have been exposed to pesticides.

For further research, one could conduct an experiment on the use of pesticide on soil compared to the use of herbicide on soil to see which one has the greater impact on the number of protozoa in the soil. One could also conduct an experiment on whether the amount of pesticide applied to the soil increases or decreases the number of protozoa in the soil. If someone wanted to look even deeper, then they could test the effects of pesticides on the amount of nitrogen in the soil to see if pesticides affect the ammonification and assimilation processes of the bacteria in soil.

References

Brock, D. L. (2015, June). Little things. Retrieved May 24, 2016, from "The little things that run the world" soil ecology program website: http://littlethings.rpcs.org/

Department of Environmental Science, Lancaster University, UK. (2004, January). Effects of organophosphate and synthetic pyrethroid sheep dip formulations on protozoan survival and bacterial survival and growth. Retrieved May 12, 2016, from NCBI website: http://www.ncbi.nlm.nih.gov/pubmed/19712353

Department of Health and Human services. (n.d.). What are organophosphates? Retrieved May 10, 2016, from centers for disease control and prevention website: http://www.cdc.gov/nceh/clusters/fallon/organophosfaq.htm\

Gerber, C. (n.d.). How do Pesticides Kill Bugs? Retrieved May 10, 2016, from ILoveToKnow website: http://garden.lovetoknow.com/wiki/How_do_Pesticides_Kill_Bugs

Ingham, E. R. (n.d.). THE LIVING SOIL: BACTERIA. Retrieved May 10, 2016, from Soil Bacteria website: http://www.nrcs.usda.gov/wps/portal/nrcs/detailfull/soils/health/biology/?cid=nrcs142p2_053862

Ingham, E. R. (n.d.). THE LIVING SOIL: PROTOZOA. Retrieved from Natural Resources Conservation Service website: http://www.nrcs.usda.gov/wps/portal/nrcs/detailfull/soils/health/biology/?cid=nrcs142p2_053867

National Science Foundation. (n.d.). *Nitrogen & Bacteria*. Retrieved May 10, 2016, from Biosa website: http://www.biosa.com/box/soilbacteria.pdf

Seymour, N. (n.d.). *http://era.daf.qld.gov.au/1296/1/Seymore_ImpactPesticidesFertilisersSoilBiota-sec.pdf*. Retrieved April 28, 2016, from http://era.daf.qld.gov.au/1296/1/Seymore_ImpactPesticidesFertilisersSoilBiota-sec.pdf

Specific Chemical (Active Ingredient) Information. (2016, March 1). Retrieved April 28, 2016, from National Pesticide information Center website: http://npic.orst.edu/ingred/specchem.html