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I. Background

In learning about the soil ecology project, our group chose to examine the effects of erosion and runoff. We chose to look at the components of runoff and how they affect protozoa. By taking soil samples from the middle of the lower field, we would test to see if three components of runoff, namely herbicide, insecticide, and fertilizer, affect the protozoa or not.

A major threat to the health of the soil is runoff. Through research, we found that impervious surfaces (concrete, roads, etc.) actually enhance the amount of runoff in one area. Unlike grass which slows erosion and cleans the runoff water, roads have a tendency to pick up harmful substances (such as oil, fertilizers, and toxins) and tend to increase the speed of runoff into ecosystems. This could affect the health of the soil directly below the road which would be receiving the bulk of the runoff. For example, pollutants gathered may contaminate the soil, making it unhealthy for plants and other organisms. The pollutants in runoff combine to create nonpoint source pollution.

Nonpoint source pollution results from several causes and can have an extremely profound effect on ecosystems. Nonpoint source pollution, henceforth NPS, refers to the pollutants that runoff. Precipitation that lands on an impermeable surface, like asphalt, does not sink into the soil as it would naturally. Instead, it flows onto a permeable surface and is absorbed or it can flow into a body of water such as precipitation landing on a parking lot and flowing into a stream. This causes two main problems. The water picks up pollutants as it travels from the impermeable surface to the body of water. Common pollutants that runoff carries include “excess fertilizers, herbicides, insecticides from agricultural lands and residential areas, motor oil, gasoline, sediment, salt, bacteria and nutrients from livestock, pet wastes, and faulty septic systems" (EPA,1994). Urban and suburban areas produce a great deal of polluted runoff as they contain more impermeable surfaces than say a forest does, along with a large population. Urban and suburban areas produce larger amounts of many of these types of NPS due to the high population density.

Erosion is the second major problem of nonpoint source pollution. Erosion occurs when water flows over a specific place and picks up particles of it. The Grand Canyon in Arizona is an instance of this. However, a far less majestic version of this happens when there are too many impermeable man-made surfaces. Since the permeable surfaces that should naturally be there are not, the water does not sink into the ground as it should. This unabsorbed water flows through the watershed and brings more NPS and more sediment into the larger body of water. For these reasons, runoff is extremely detrimental to soil's health as it contaminates it and removes vital sediments from it.

Sometimes, one of the components in runoff is fertilizer. Fertilizers are one of the most popular ways for gardeners and landscapers to promote the health and growth of their lawns and plants. While there are many different types of fertilizers, all have three major chemicals in them. The first chemical in fertilizer is nitrogen (N), which increases the amount of foliage of the area by increasing the enzymes, which cause the chemical reactions within the plant's cells. The nitrogen within a plant promotes the protein and nucleic acid synthesis. Protein synthesis is one of the four major tasks of each cell therefore, nitrogen is fundamental. Nitrogen is such an imperative compound to amino acids, that without it, amino acids cannot be formed. The amino acids are what make up the proteins in a cell. Without amino acids, the proteins cannot be formed. Proteins, specifically enzymes cause the making and breaking of bonds (the reactions) between the chemicals of the cell: lipids, proteins, carbohydrates, nucleic acids, and water. Without amino acids, there are no proteins, and without the proteins, there are no enzymes. If there are no enzymes to cause chemical reactions in the cell, there is no way for the cell to perform its form major tasks, and because the cells die, the plant dies. Therefore, nitrogen is a vital component of fertilizer for optimum growth of plants.

The next major chemical in fertilizer is phosphorus (P), which strengthens the roots of the plant by aiding in the formation of DNA, which is then copied into RNA. RNA is used to create specific proteins known as enzymes, which control the reactions between the proteins, lipids, carbohydrates, nucleic acids, and water, which are the main chemicals of the cells in the plant. Phosphorus is also vital in building and processing ATP molecules, which are necessary for a plant to complete cellular respiration (or transference of energy). For cellular respiration to occur, the ATP (made up of three phosphates), takes one of its phosphates and attaches it to a protein. This causes the protein to change shape, which allows energy to be transferred. As the phosphate then detaches itself from the protein, the protein goes back to its original shape. Each time one of the phosphates is added or taken away from the protein it causes the protein to transform from one contour to the next, therefore work (energy) is being completed. Without energy, the plant would not be able to complete cellular respiration or its growth.

The last major chemical in fertilizer is potassium (K) which reinforces the immune system of the foliage on which the fertilizer is being applied. Along with the three major chemicals, most fertilizers also have Magnesium, Calcium, Sulfur, Iron, Manganese, Zinc, Boron, and Molybdenum. These less prevalent chemicals are added to the fertilizer to prevent it from gathering into chunks.

Insecticide is the second chemical we will be using. Insecticide is a type of pesticide specifically used for the elimination of insects. Insecticide not only kills insects on plants, but inside the soil as well. Residual insecticide kills by leaving an amount of poison on a surface that the insect will contact (therefore poisoning the insect). Contact insecticide is sprayed directly on the insect, killing it instantly. Stomach poisons are special insecticides that can be eaten by the insect. The insecticide enters the stomach and is absorbed by the rest of the body. By spraying insecticide on soil, the substance can remain on the soil. This alters the amount of protozoa growing, making it increase or decrease. By testing, we will be able to see what the insecticide causes the protozoa to do.

For our experiment, we will be using the insecticide Malathion. Malathion tends to be broken down rapidly in soil within 1 to 25 days (Malathion, 1996). Malathion can last for three weeks in distilled water, which is essential to collecting the data (Malathion, 1996). Although it can easily move through the soil's profile, it is highly unlikely to contaminate ground water due to its low persistence. Breakdown is caused by a combination of biological degradation and non-biological reaction with water. The bacteria degrade the insecticide easily because it does not stick to the soil. The break down is called enzyme-catalyzed hydrolysis.

Along with choosing to add a fertilizer and an insecticide to soil samples, we chose to examine the effects that an herbicide would have on protozoa. Herbicide is a type of pesticide specifically used for the elimination of plants. There are a few different types of herbicides. Root mitotic inhibitors block cell division in plants, so the "root development is inhibited (root pruning) which leads to plant stunting." This could affect protozoa in the long run because there would be less material for decomposition, therefore allowing for less material for the protozoa and bacteria to eat. Pigment inhibitors "contribute to chlorophyll destruction." Shoot inhibitors "affect cell growth and division and stunt plant shoots and cause leaf crinkling." Photosynthetic inhibitors "interfere with photosynthesis by blocking electron transfer, resulting in damage to the plant membranes and cell death" (How Herbicide Works, 1998). This could also affect the amount of food getting to the protozoa, eventually decreasing the population. Herbicides most likely affect only a small amount of protozoa directly, but they do, however, affect the food and habitat of other organisms by killing off the plants (Soil Biology and Land Management, 2004). If the plants that eventually decompose are eliminated, there is less and less food sources for the protozoa. The herbicide either absorbs into the soil or runs off into the water bodies near by. The herbicide that is absorbed into the soil usually has a pH level close to the soil. The content of herbicide that has a pH level close to the soil is retained more strongly and therefore runoff and erosion do not affect it as much. When it is the opposite (the pH level of the herbicide is not close to the soil), runoff and erosion affect it more. They are more susceptible to plant uptake. The herbicide molecules easily bind themselves to particles in clay, but do not attach for a long period of time (Herbicide Behaviors in Soil, 2004). Herbicide, insecticide and fertilizer will have either a direct or indirect affect on protozoa in soil.

Protozoa play an integral role in soil ecology and are therefore an excellent indicator of a specific soil's overall health. Protozoa are unicellular animals that dwell in all manner of environments that contain enough water to allow them mobility, but we will be focusing on soil-dwelling ones, as they are the only kind that directly relate to our experiment. They primarily consume bacteria, but can also eat “protozoa, soluble organic matter, and sometimes fungi” (Soil Protozoa | NRCS SQ, 2005). There are three major types of protozoa: ciliates, amoebae, and flagellates. Ciliates, the largest in physical size of all protozoa, move using cilia, hence the name, and include such species as *vorticella*. Amoebae, the second biggest group of protozoa, move by forming temporary pseudopods to locomote. There are two types of amoebae, testate amoebae like *difflugia corona*, which have an exterior covering and naked amoebae like *amoeba proteus* (Amoeba, 2005), which have no such covering. Flagellates, the smallest type of protozoa, move using whip-like tails to propel themselves (Soil Protozoa | NRCS SQ, 2005) and include such species as *giardia lamblia*.

Protozoa mainly exist “near the soil surface, especially in the upper 15 cm” (Protozoa, 2000). In certain extremely fertile soils, there can be approximately 1,000,000 protozoa per 4.7 grams of soil whereas, in less fertile soils, a sample of the same mass can sometimes contain only 1000 protozoa (Soil Protozoa | NRCS SQ, 2005). The kinds of protozoa in a specific soil depend on the soil’s makeup. For instance, soils containing high levels of clay tend to have correspondingly high populations of smaller protozoa whereas soils with low levels of clay have high populations of larger protozoa (Soil Protozoa | NRCS SQ, 2005). The reason soils with higher clay contents tend to have larger populations of small protozoa is because clay has smaller particles than other materials that compose soil i.e. less space between particles for the protozoa to live and therefore this environment suits smaller protozoa better than it would larger protozoa.

Protozoa provide nutrients such as NH^{+4} by mineralizing them or in simpler terms "making them available for use by plants and other soil organisms" (Protozoa, 2000). In some environments, microinvertebrates, such as nematodes, eat them (Protozoa, 2000). Therefore, protozoa provide nutrients necessary for plants to form amino acids, which in turn form proteins, etc. When plants are able to perform these vital tasks, they provide in turn provide nutrients for primary consumers on the food chain and so on. As the various organisms die, protozoa along with other decomposers recycle their nutrients back through the cycle of production and consumption. Thusly, protozoa are incredibly important to soil ecosystems and a good measure of soil's wellbeing. Since they are so vital to soil health, our group decided to test out the effects of different components of NPS upon them. This in turn shows the damage NPS inflicts on general soil health as they are indicative of soil's health.

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II. Lab Outline

Problem: Do herbicide, insecticide, and fertilizer, when applied to the soil, decrease the population of protozoa in the soil?

Hypothesis: Herbicide and insecticide will decrease the amount of protozoa in the soil, but fertilizer will increase the amount.

Experiment:

Independent Variable: Miracle-Gro fertilizer (40 microliters), Round-Up herbicide (40 microliters), or Malation insecticide (40 microliters) applied to the soil (affecting the protozoa)

Dependent Variable: number of protozoa in a cubic centimeter of soil

Negative Control: soil without herbicide, insecticide or fertilizer added

Controlled Variables:

- Time of sampling
- Chemicals already on the field
- Amount of light protozoa cultures exposed to
- Time between soil sample taking
- Amount and strength of herbicide, insecticide and fertilizer
- Depth of soil sample and diameter of soil cylinder (15 cm by 2.3cm)
- Weather conditions of soil sampling
- Location of soil sampling
- Type of herbicide, insecticide and fertilizer
- Amount of drying time for soil
- Amount of soil dried
- Conditions of Petri dishes
- Type of Petri dish
- Size of Petri dish
- Amount of time the soil sits after the chemicals are added (48 hours)
- Amount of water used to rehydrate
- Amount of each chemical added
- Amount of 2^{nd} filtrate placed on microscope slide
- Number of drops of methyl-green stain (7 drops) placed on microscope slide
- Type of plastic baggie
- Amount of time the chemical and soil are mixed together
- Size and type of microscope slides
- type of plastic cup
- type of screen or mesh
- strength of microscope
- method of counting

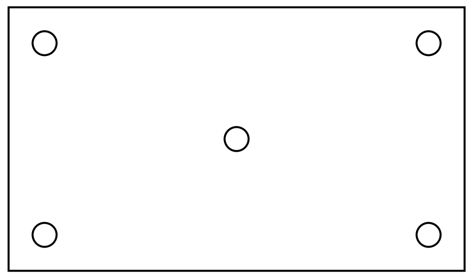
- same equation each time
- type of scale
- amount of sifted soil

Step-by-Step Procedure:

1. At N 39.35865, W 76.63718, take 12 samples of soil that are 15 centimeters deep and 2.3 centimeters wide at the same time on the same day.
2. Take the samples using the soil extractor.
3. Insert the soil extractor into the ground and twist until the ground reaches the first line, which is 15 cm. Once the ground has reached the first line, twist the extractor out of the ground.
4. Remove the soil from the extractor using a finger and put it into a clean, plastic baggie. Each soil sample should be in its own plastic baggie. Label each bag with the chemical you will add to that sample.
5. Take each bag and place each 15 cm sample of soil into the bottom of a separate, clean, empty Petri dish; and allow the 12 samples to dry completely for 48 hours.
6. Put each soil sample in a separate plastic cup and rubber band a 1 mm^2 nylon screen or mesh on the top of the cup.
7. Put the bottom of a new Petri dish on the scale and re-zero it.
8. Sift 10 g of each soil sample into a 2nd clean Petri dish (one dish per sample) by shaking the soil directly into the bottom of the Petri dish.
9. Add 20 ml of distilled water to each Petri dish to saturate the soil.
10. Cover the Petri dish with its lid and allow to sit for 7 hours. If the experiment cannot be continued exactly after 7 hours, put the samples in the refrigerator until the experiment can be continued.
11. Pour a very small amount of herbicide into one plastic cup. Pour a small amount of insecticide into another plastic cup. Pour a small amount of fertilizer into a third plastic cup.
12. Set a micropipette to 40 microliters and add 40 microliters of the herbicide to the first soil sample.
13. Stir the herbicide into the soil for 15 seconds.
14. Each time you add 40 microliters, make sure you have gotten a new tip and put the old one in sterilizing solution.
15. Repeat steps 12 to 14 for two new soil samples.
16. Set those three samples aside and begin with three new soil samples.
17. Repeat steps 12 to 16 for insecticide.
18. Repeat steps 12 to 16 for fertilizer.
19. The last three samples are the negative controls.
20. Let the soil samples sit for 48 hours.
21. Place each soil sample in a separate modified Uhlig extractor containing 30 ml of distilled water for 24 hours.
22. Remove the filtrate of each chemical and filter each a 2nd time using new 12.5 cm qualitative filter paper
23. Using a capillary tube, deposit 7 µl of methyl-green stain on a clean microscope slide (1 µl = 1 drop from capillary tube). Use a disposable graduated Beral-type

pipette to add 18 µl (the first demarcation on the pipette) of the 2^{nd} filtrate to the stain on the microscope slide and cover with an 18 x 18 mm^2 cover slip.

24. Examine under a light microscope at 40x. You will examine 5 spots on the slide (as seen below) and count the number of protozoa in each of the five spots and then average all five.



25. Take the average you calculated in the previous step and plug it into the following equation to find the number of protozoa per gram of soil.
26. Use the following equations to determine the population density of protozoa in the soil sample:
    [(# per field of view at 40x) • (total ml of 2^{nd} filtrate) • 747] ÷ (grams of sifted soil) = # of protozoa per gram of soil[*]

Data/Observations

<u>Trial 1</u>

	With herbicide	With fertilizer	With insecticide	Negative Control
Number of protozoa per gram of soil	197,955	89,640	129,231	152,388

<u>Trial 2</u>

	With herbicide	With fertilizer	With insecticide	Negative Control
Number of protozoa per gram of soil	599,841	67,230	298,053	122,508

[*] Steps 5 – 26 from Kate Brockmeyer

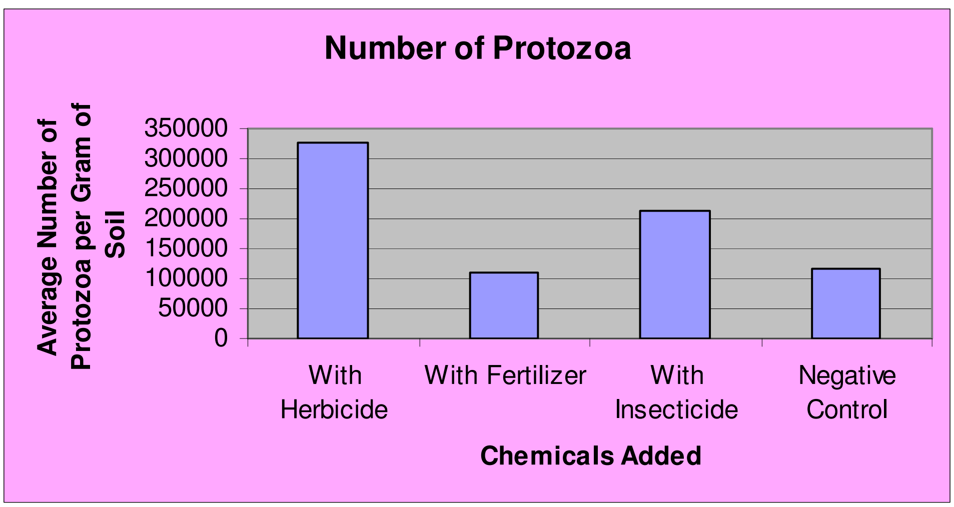
Trial 3

	With herbicide	With fertilizer	With insecticide	Negative Control
Number of protozoa per gram of soil	179,280	173,304	Data point eliminated	73,206

Averages

	With herbicide	With fertilizer	With insecticide	Negative Control
Number of protozoa per gram of soil	325,692	110,070	213,642	116,046

Analysis



As can be seen from the above graph, herbicide had the highest protozoa count, insecticide had the second highest, negative control had the third highest and fertilizer had the lowest protozoa count.

III. Conclusion

Our hypothesis, stating that fertilizer would cause the protozoa count to increase, while herbicide and insecticide would cause the protozoa count to decrease, proved to be incorrect. Instead of fertilizer increasing the protozoa count, it actually caused the protozoa count to decrease while herbicide caused the protozoa count to increase. After averaging all data from our three trials, we found that the soil we added herbicide to had the greatest protozoa count: 325,692 protozoa per gram of soil. The insecticide averaged the second highest at 213,642 protozoa per gram of soil. The fertilizer average of 110,070 protozoa per gram of soil was lower than our negative control average of 116, 046 protozoa per gram of soil. This shows that fertilizer caused the number of protozoa to decrease contrary to our hypothesis. However, as said in the background, Malathion (our brand of insecticide) is easily degraded and broken down by bacteria inhabiting the soil. This factor could have contributed to some of the experiment's results involving insecticide.

There is another main reason for the results to be the opposite of our hypothesis. Insecticide is used to eliminate insects while herbicide is used to control invasive plant growth. Though the two chemicals target different areas, they both affect protozoa in the same way. Because herbicide and insecticide increase the amount of decaying matter, the bacteria in the soil have more food to consume. Therefore, the population of the bacteria increases. Since there are more bacteria, then there is more food for the protozoa, causing the number of protozoa to increase.

There is also an explanation for the decrease of protozoa when fertilizer was added. The waste products released by the bacteria in the soil, are used by plants as nutrients. These waste products are the same as the three main chemicals in fertilizer: nitrogen, phosphorus, and potassium. When fertilizer is added to the soil, there is an increase of these chemicals, causing the plants to flourish. However, because nitrogen, phosphorus, and potassium are considered 'waste' for the bacteria, an increased amount of them would cause the bacteria to die. Since the bacteria are the major food source for the protozoa, if the number of bacteria decreases, then the protozoa will not have as much food to eat. Therefore, the protozoa population would decrease as well, explaining why the protozoa count (when fertilizer was added) was lower than the negative control.

The only problem that we ran into during our experiment was when one sample of our soil got contaminated. When we were adding 20 milliliters of distilled water to each sample (step 10 in our procedure), the sample got bumped, causing some of the soil (with water added) to be spilled over the side of the Petri dish. This solution (data point of insecticide in trial 3) was therefore contaminated and had to be dropped from our experiment and data. This affected our average protozoa count for insecticide, making it lower than the others.