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Which Mulch Is Best For the Protozoa? Background paper

Mulch is often used by people in gardens, on paths, and even on playgrounds. Mulch can give an area a more appealing appearance, but it does much more than improve the look of a garden. It can be extremely helpful in maintaining the health of the soil. Simply by covering the soil, mulch prevents crusting of the soil, thereby improving absorption, and water movement. (Kluepfel, 2004) With mulch, soil is able to hold moisture better because mulch provides a shield, preventing a majority of the water underneath it from evaporating. (Flavel, 2001). Erosion is also lessened because when rain falls, the mulch, again, acts as a shield and prevents the soil from being compacted, or worn away. The mulch, since it creates a layer on top of the soil, prevents soil splashing when it rains. This helps control erosion and minimizes runoff. Furthermore it prevents soil-borne diseases from splashing onto the plants. (Kluepfel, 2004) Mulch also prevents weed growth for the most part by preventing the seeds from germinating. If the mulch is applied to a weed free surface, there will not be enough light for weeds that have not begun to grow to germinate. (Evans, 9/2000) Also, very importantly, mulch maintains a constant temperature as it prevents a lot of heat from the sun entering or leaving the soil. (Williams, 7/96)

To get a deeper understanding of how mulch is useful, you have to look into the aspects that you cannot see. For example, mulch can alter the pH of the soil to be appropriate for the plants that grow there. (VanDyk, 1998). Most people know that pH is the level of acid or base in a solution. Although this is true, there is more to pH than just acidity. The pH scale is based on the concentration and activity of hydrogen ions (H+). The scale centers around water as the neutral. Water contains an equal amount H+ and

OH- ions. This creates a pH of 7. An acidic solution contains more H+ than OH- ions, and creates a pH of 0-7. If a solution has more OH- than H+ ions, it is alkaline and has a pH of 7-14. (Unknown, 2002)

The pH is important in the soil because certain plants cannot grow in certain pH levels. If the soil pH is above 7 then it is referred to as alkaline and if it is below 7 then it is called acidic. Plants have a hard time growing in either extreme but can grow in both mild acidic and mild alkaline soil. The best pH levels would be 6 to 7.5. This is the pH level where the essential nutrients for the plants are most available. (VanDyk, 1998) The pH also affects the nutrients in the soil. For example, phosphorus is only available to plants at a pH of 6.0 to 7.0. Nitrogen is available at a pH as low as 5.5 but the bacteria are able to function better at a higher pH level. (Williams, 7/96)

The pH can affect the solubility of nutrients in the soil. The H^+ ions in an acidic pH react with any OH^- ions in the soil. This reaction creates H_2O, and uses up the OH^- ion so the nutrient that contained the H^- ion, now does not have it anymore, so it produces more. These react once again with the H^+ ions from the acid pH creating more H_2O (water) and making the nutrient more soluble. A base solution can have the same effect if the nutrient has H^+ ions in it because the OH^- ions in the base solution would still react and make H_2O. (Ms. Lentz, 5/25/04, “conversation”)

Another important aspect of the soil is microscopic. The protozoa that live in the soil play a major role in the overall health of the soil and the plants that grow in it. The first step in understanding why protozoa are so important is to understand what they are. Protozoa are grouped into three types based on shape. Ciliates are the largest kind. They move by hair-like cilia. They eat the other two types of protozoa as well as the bacteria

in the soil. Amoebae are the second largest protozoa. They transport themselves using a temporary extension like a foot or sometimes referred to as a "pseudopod, and are further divided into two subtypes; testate amoebae, which have a shell covering around them, and naked amoebae, which do not have a shell covering them. The third and smallest group of protozoa is the flagellates. These small protozoa use whip like flagella to move through the soil. (Ingham April 2004). All these different kinds of protozoa essentially do the same thing. That is, they eat the bacteria and release the nitrogen in them for the soil to use.

Along with mulch, the protozoa in the soil help maintain the nutrients and are partly responsible for the nitrogen cycle in the soil. The nitrogen cycle is important not only in the soil, but also Earth as a whole as nitrogen makes up 80% of the atmosphere. (Campbell, 2004) Nitrogen is used in plants to make proteins, hormones and nucleic acids. Proteins are extremely important because they help with all the 4 tasks of cells; chemical reactions, hormone receptors, and DNA translation, and reproduction. Hormone proteins play a major role in reproduction. Nucleic acids are responsible for cellular energy, the genetic code, and the RNA that translates the genetic code are both made of nucleic acids. (Tamarakin, May 19, 2004) The plants cannot get the nitrogen they need to survive directly from the atmosphere. N_2 is atmospheric nitrogen and plants cannot use it so as it gets into the soil, it goes through nitrogen fixation so it is accessible to the plants. (Campbell, 2004) The N_2 in the air goes underground where the ammonifying bacteria change it into ammonia (NH_3). Ammonia also gets into the soil through animal waste. The nitrogen-fixing bacteria change the N_2 into ammonium (NH+4), which is then processed into nitrate (NO^{-3}), which the plants are able to use

through their roots. (Campbell, 2004) Although it is mainly the bacteria that process the N_2 into nitrate for the plants, the protozoa are the ones that get the nitrate from the bacteria so it can be used by the plants. (Ingham, Apr. 26, 2004). The protozoa survive by eating the bacteria in the soil. Since the bacteria have more nitrogen in them than the protozoa need, the protozoa excrete extra nitrogen into the soil. This essential contribution to the nitrogen cycle is often neglected. The protozoa also help maintain the bacteria in the soil at a reasonable level. They eat the bacteria and are also a major food source for other organisms in the soil such as microarthropods and earthworms. (Ingham Apr 26, 2004) Protozoa also help suppress disease by eating or competing with pathogens in the soil. (Ingham, April 2004) These protozoa are quite important but unfortunately do not get the credit they deserve all the time.

Environmental factors affect the amount and variety of protozoa in the soil. Soil in forests which are fungal dominated tend to have more testate (shelled) amoebae and ciliates than other types. Soils with a lot of bacteria have flagellates and naked amoebae mostly. Soils with high clay content tend to have smaller protozoa like the flagellates and naked amoebae. Those soils with a coarse texture have a variety of protozoa. They tend to have the larger flagellates and both kinds of amoebas and the ciliates as well. (Ingham, April 2004) The pH level of soil can change the density of protozoa as well. With an alkaline (high) pH, the density of microbes in soil is decreased along with the density of protozoa. In soil with an acidic (low) pH, density of microbes in the soil is decreased because of lower micronutrient availability.

Although we know that mulch is helpful to the health of soil, we do not know which mulch provides the best pH for the protozoa in the soil and therefore is best for the

soil and the plants growing in it. At Roland Park, we use a number of different mulches to surface our soil. On the flowerbeds, we use triple hardwood shredded mulch, and on the trails in the back woods, we use chippings from the poplar, oak, and pine trees that have been pruned or cut down. (Whalen Jennifer, May 1, 2004 "conversation"). We opted to test the relationship between the pH of different kinds of mulch around the Roland Park campus and the amount of protozoa in the soil based on the pH of the mulch. This will potentially show us which mulch is best to use on our campus and could be very helpful for the landscaping staff to know. After researching, we have hypothesized that the mulch from the poplar, oak, and pine trees will generate an optimal amount of protozoa for the soil at our school. We figure that the health of the soil was influenced by the number of protozoa as well as other effects of the pH level, and can be shown by the plant life around the mulch. The mulch from the poplar, oak, and pine trees is the mulch that is used in the back woods, which was observed with an abundance of plants growing near it and therefore would seem to have healthy soil.

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Procedure:

Problem: Which type of mulch used at Roland Park (triple hardwood shredded mulch and chippings from poplar, oak, and pine trees mulch) generates the pH level for maintaining the optimal density of protozoa in the soil?

Hypothesis: The chippings from poplar, oak, and pine trees mulch will generate the optimal conditions for protozoa in the soil.

Procedure:

Variables:

-Independent: pH level of solution applied to soil

-Dependent 1: Density of protozoa in the soil

-Dependent 2: pH level of soil

Controls:

-Negative control 1: density of protozoa and pH level of soil prior to adding excess pH solution

-Negative control 2: plot labeled Negative control which does not receive any excess pH solution

-Control variable list: amount of soil collected for testing, water is distilled, amount of water used to saturate soil during protozoa process, test for protozoa and pH at the same time, time between pouring pH solution and testing for protozoa, amount of water used in Uhlig extractor, shouldn't rain between the time the pH solution is poured and data is collected, length and width of test plots, amount of pH solution poured onto test plots, both pH solutions are poured onto the test plots at the same time, amount of methyl green stain on each microscope slide, magnification of microscope when viewing protozoa, amount of liquid on each microscope slide, size of cover slip, amount of time soil saturates, amount of time soil sample is in Uhlig extractor

Step by Step:

1. Find an area where triple hardwood shredded mulch is used and find an area where chippings from poplar, oak, and pine trees are used as mulch
2. Take three (15cm deep by 2cm in diameter) samples of the soil under the triple hardwood shredded mulch and place each sample in a separate labeled plastic bag
3. Take three (15cm deep by 2cm in diameter) samples of the soil under the chippings from poplar, oak and pine trees that is used as mulch and place each sample in a separate labeled plastic bag

4. Test the pH level of the soil in each bag using the LaMotte STH Series process for pH testing and record each pH level
5. At 39.35800 ^{o} N and 76.63632^{o} W mark six (15 centimeters by 15 centimeters) plots with flags, one at each corner. The first row is trial 1. In trial 1, there are three plots. The first plot is the negative control (label as such), the second plot is plot 1 (label as such) and the third plot is plot 2 (label as such). The second row is trial 2. In trial 2, there are three plots. The first plot is the negative control (label as such), the second plot is plot 1 (label as such) and the third plot is plot 3 (label as such). This is for a total of 6 plots, each 15 centimeters by 15 centimeters. The layout should look like:

Negative control (trial 1)	Plot 1 (trial 1)	Plot 2 (trial 1)
Negative control (trial 2)	Plot 1 (trial 2)	Plot 2 (trial 2)

6. From each plot, take three (15cm by 2 cm in diameter) soil samples using a soil core test sample. Take the samples in a triangle shape, (one sample at the top and two at the base). Place each soil sample into its own-labeled plastic bag.
7. Put each soil sample into a separate Petri dish and label it. Allow each Petri dish with soil sample to sit out for 24 hours to dry completely.
8. Find the number of Protozoa present in each dried soil sample and record the number of protozoa. To do so, follow the procedure below: *Cited*

a. Sift 9-10 g of the dried soil from step 7 into a 2nd clean Petri dish using a 1 mm^2 nylon screen or mesh.
b. Using the leftover dried soil of each sample (that was not sifted into the second Petri dish) find the pH of each soil sample by following the LaMotte STH Series process for pH testing.
c. Add 20 ml of distilled water to saturate the soil
d. Cover the Petri dish with its lid and allow to sit for 7 hours
e. Place the soil sample in a modified Uhlig extractor containing 30 ml of distilled water for 24 hours. Then immediately place each soil sample in the refrigerator as to lower the protozoa's metabolism so that they do not reproduce.
f. Remove the filtrate and filter a 2nd time using 12.5 cm qualitative filter paper. Then immediately place each soil sample in the refrigerator as to lower the protozoa's metabolism so that they do not reproduce.
g. Using a capillary tube, deposit 7 µl of methyl-green stain on a clean microscope slide (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 step e to the stain on the microscope slide and cover with an 18 x 18 mm^2 cover slip.
h. Examine under a light microscope at 40x in 5 different field of views count the number of protozoa in each 2nd filtrate. Then average the five numbers in each 2nd filtrate and put each average into the equation of step i.

i. [(# per field of view at 40x) · (total ml of 2nd filtrate) · 747] / (grams of sifted soil) = # of protozoa per gram of soil

Cited: Kate Brockmeyer, *A New Method for Soil Protozoa Extraction and Population Estimation*.

9. Create a pH solution with the same pH level as the soil you had collected under the triple hardwood shredded mulch. Do this by repeatedly mixing sodium hydroxide with water until the solution reaches the pH level of 7.3.
10. Create a pH solution with the same pH level as the soil under the chippings of the Poplar, Oak, and Pine trees mulch that you had collected. Do this by repeatedly mixing hydrochloric acid with water until the solution reaches the pH level of 6.73.
11. Pour 1 liter of the 7.3 level pH solution onto Plot 1 in Trial 1 and another 1 liter of the 7.3 level pH solution onto Plot 1 in Trial 2. At the same time as pouring the 7.3 solution, also pour 1 liter of the 6.73 level pH solution onto Plot 2 of Trial 1 and pour another 1 liter of the 6.73 level pH solution onto Plot 2 in Trial 2.
12. Do not put any pH solution onto the plot labeled Negative Control in either Trial 1 or Trial 2
13. After 48 hours, take three (15cm by 2cm in diameter) samples from each plot (including the negative controls). Take the samples in a triangle shape, (one sample at the top and two at the base). Put each soil sample into its own-labeled plastic bag.
14. Repeat steps 7-8 with the soil samples collected from step 13.

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Data and Analysis:

Trial:Plot:Sample	Soil pH before	Protozoa/gram before	Trial:Plot:Sample	Soil pH after	Protozoa/gram after
T1 NC 1	6.4	35761	T1 NC 4	6.8	86252
T1 NC 2	6.8	84609	T1 NC 5	6.7	124246
T1 NC 3	6.6	53469	T1 NC 6	6.6	44518
T2 NC 1	6.6	49287	T2 NC 4	6.8	66999
T2 NC 2	6.6	73111	T2 NC 5	6.6	250779
T2 NC 3	6.8	55447	T2 NC 6	6.6	51111
T1 P1 1	6.8	73390	T1 P1 4	6.9	41044
T1 P1 2	6.6	65459	T1 P1 5	6.9	30034
T1 P1 3	6.8	33459	T1 P1 6	7.0	128273
T2 P1 1	6.5	11099	T2 P1 4	6.9	150203
T2 P1 2	6.6	80204	T2 P1 5	6.9	160071
T2 P1 3	6.8	73914	T2 P1 6	6.8	88355
T1 P2 1	6.6	56422	T1 P2 4	6.8	77084
T1 P2 2	6.6	93722	T1 P2 5	6.8	78511
T1 P2 3	6.4	81777	T1 P2 6	6.8	77813
T2 P2 1	6.7	65264	T2 P2 4	6.6	166519
T2 P2 2	6.6	32582	T2 P2 5	6.7	91999
T2 P2 3	6.7	113639	T2 P2 6	6.5	92371

Plot	Average soil pH before	Average protozoa/gram before	Plot	Average soil pH after	Average protozoa/gram after
Negative Control	6.6	61676	Negative Control	6.7	103984
Plot 1	6.68	73421	Plot 1	6.9	99663
Plot 2	6.5	73910	Plot 1	6.7	97383

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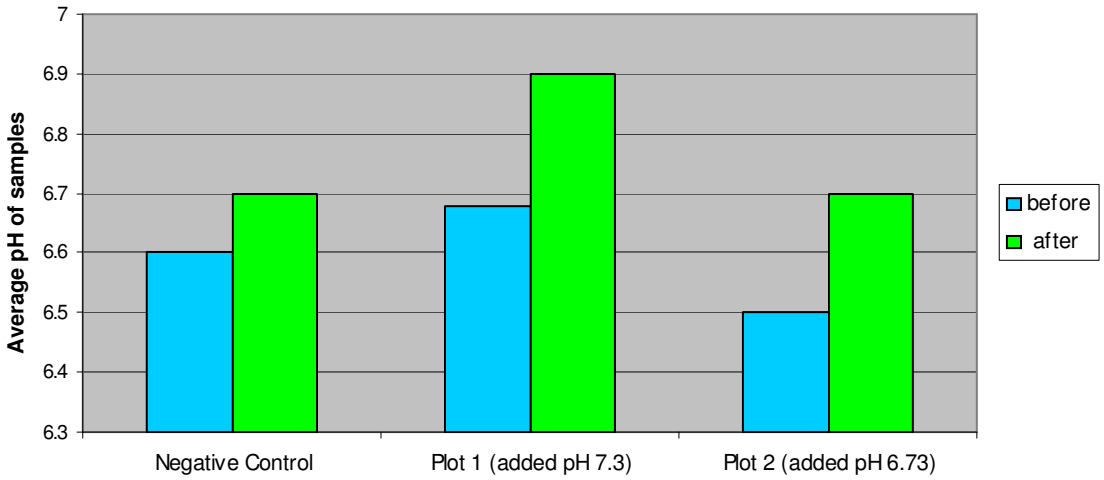
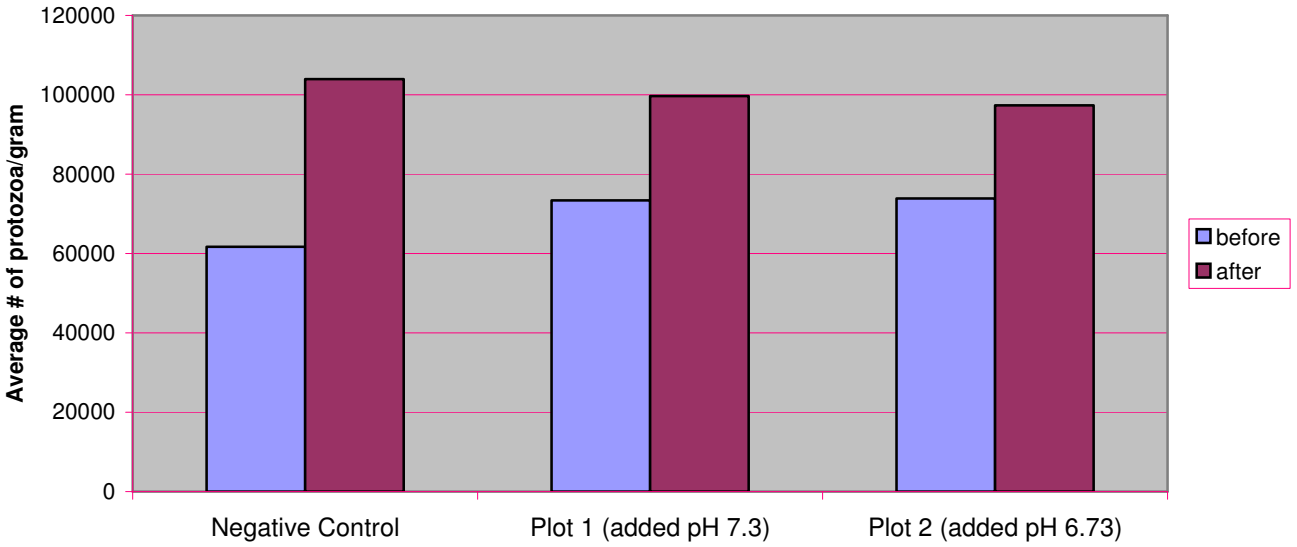
pH Level Before the pH Solution of the Mulch was Delibrately Added and pH level After the pH Solution of the Mulch was Delibrately Added



Protozoa Density Before Before the pH Solution of the Mulch was delibrately Added and the Protozoa Density After Before the pH Solution of the Mulch was Delibrately Added



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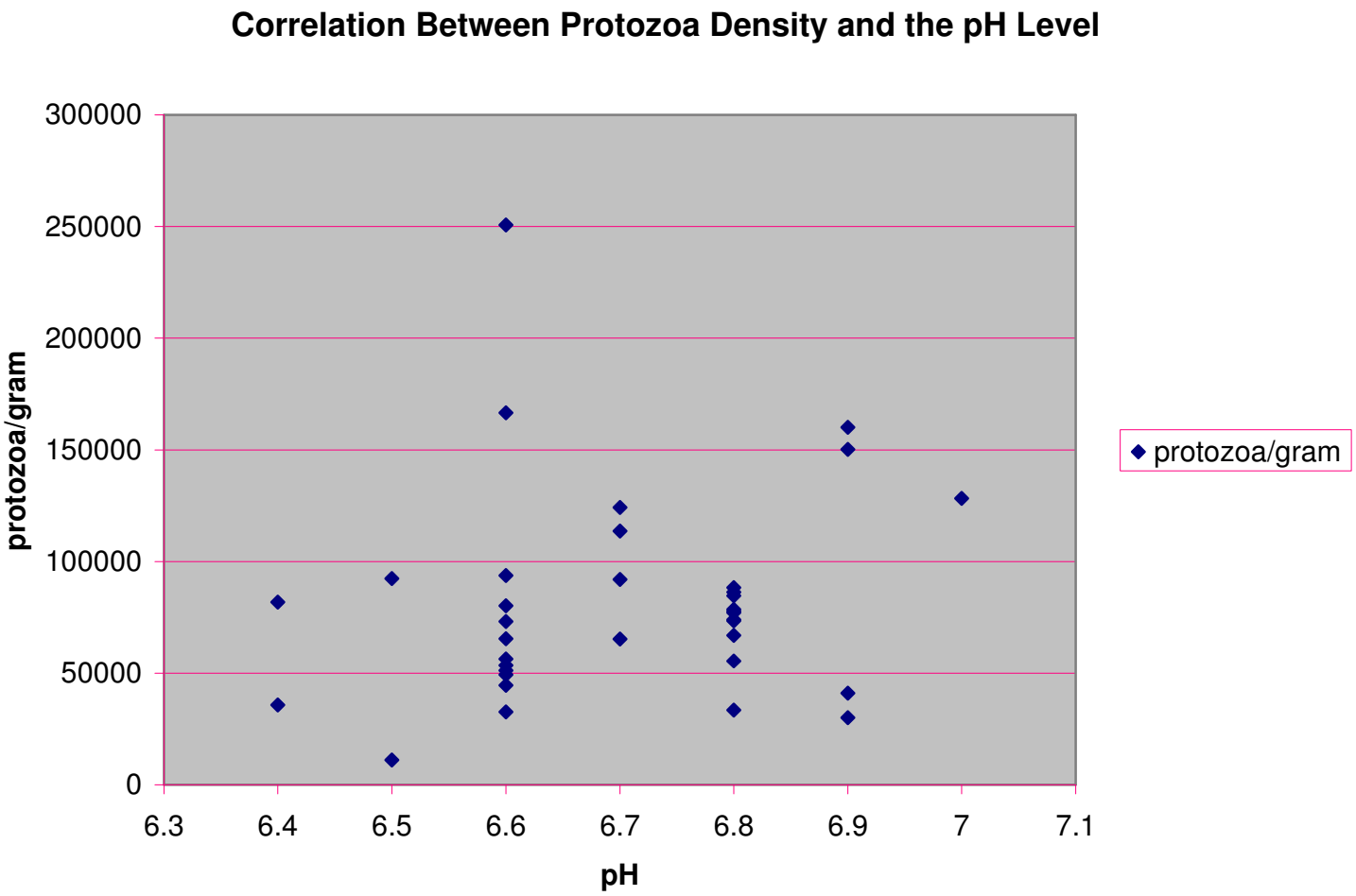

Analysis:

As can be seen in the graphs above, a corrected analysis must be calculated. Because the negative controls of protozoa density and pH levels both changed, the change in each must be subtracted from each plots' data from the data for after the pH solutions were added. In the protozoa graph, the negative control corrected value for the data for after should be equal to what it was before the pH solution was added to the other plots. Therefore 42,308 must be subtracted from both plots one and two for the data of after pH solution was added. In plot one, the corrected average number of protozoa per gram of soil after the ph solution of 7.3 was added is 57,355. In plot two, the corrected average number of protozoa per gram of soil after the pH solution of 6.73 was added is 55,075. In the pH graph, the negative control corrected value for after should also be equal to that of the before data. Therefore, 0.1 must be subtracted from both plots one and two for the data after the pH solutions were added. In plot one, the corrected average pH level after

the pH solution of 7.3 was added to it is 6.8. In plot two, the corrected average pH level after the pH solution of 6.73 was added to it is 6.6.

Conclusion:

From the data collected, it is evident that our hypothesis, the chippings from poplar, oak, and pine trees mulch generates the optimal conditions for protozoa in the soil, is correct. We added a solution with a pH of 7.3 to plot 1, representing the pH of the chippings from poplar, oak, and pine trees mulch and we added a solution with a pH of 6.73 to plot 2, representing the pH of the triple hardwood shredded mulch. In both cases, the pH level rose from its original pH level after the pH solutions had been added. The average pH level of plot 1 was 6.68 before the solution was added and rose to 6.9 after the pH solution of 7.3 was added. The average pH level of plot 2 was 6.5 before the pH solution was added and rose to 6.7 after the pH solution 6.73 was added. It must be taken into consideration however, that the negative control also increased over the 48-hour period. The average pH level of the negative control plot before the solutions had been added to plot 1 and plot 2 was 6.6 and rose to 6.7 after the pH solutions were added to plots 1 and 2. If this change in the negative control is used to correct the data, then the corrected pH level of Plot 1 changes from 6.68 to 6.8. The corrected pH level of plot 2 changes from 6.5 to 6.6. Even with the corrected values, it is still evident that the pH level of plot 1 was .2 higher than that of plot 2.

The protozoa density of each plot was altered by the change in the pH levels. The average density of protozoa in plot1before the pH solutions were added was 73,421 protozoa per gram of soil and the protozoa density then rose to 99,663 protozoa per gram of soil after the pH solution of 7.3 was added to plot 1. This increase was more than that

of plot 2. In plot 2, the average density of protozoa per gram of soil before the pH solution was added to it was 73,910 and then rose to 97,383 protozoa per gram of soil after the pH solution of 6.73 was added to plot 2. Because the pH solution with a pH level of 7.3 was added to plot 1 and a pH solution with a pH level of only 6.37 was added to plot 2, it is proven that the plot with a higher pH level obtained a higher average density of protozoa. The change in the negative control must be taken into account. With corrected data, the protozoa level in plot 1 began at 73,421 protozoa per gram of soil. Then the protozoa density decreased to 57355. In plot 2, where the 6.73 solution was added, the protozoa density began at 73,910 and decreased to 55075 after the solution was added. In both plot 1 and plot 2 the protozoa densities decreased. However, in plot 1, with the corrected data, the average number of protozoa per gram of soil was 57355 after the pH solution of 7.3 had been added to it. In plot 2, with the corrected data, the average number of protozoa per gram of soil was 55075 after the pH solution of 6.73 had been added to it. Plot 1's average number of protozoa per gram of soil was 2280 higher than that of plot 2's.

As seen in the chart showing correlation between average pH levels and protozoa density, it can be seen that as the pH levels rises, so does the protozoa density per gram of soil. This further proves our hypothesis because the mulch from poplar, oak, and pine trees has a higher pH level than the Triple Shredded Hardwood mulch. Therefore the mulch from the poplar, oak and pine trees provide the soil with the optimal pH level for the protozoa.

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