SOIL ECOLOGY PROJECT 2013

By: Molly Baker, Claire Evans, and Julianna Rak

Molly Baker, Julianna Rak, and Claire Evans

Mr. Brock

Soil Ecology Project

30 May 2013

Background

Single celled organisms, including bacteria, fungi and protozoa, flourish all over the earth's surface. These microbes provide essential services to other living organisms by decomposing waste and forming nutrients (Fredrickson and Onstott, 1996), and they exist in large numbers in the soil as long as a source of carbon, such as dead plants, is present. Without the energy and nutrients from the microbes, the larger producers, primary consumers and secondary consumers in an ecosystem would not be able to survive, and therefore soil microbes are the foundation of every ecosystem because all other parts depend on them.

One specific type of microbe, bacteria, plays an especially critical role in helping sustain life in the soil. The smallest and most abundant microbe found there, each of the different species of bacteria falls into one of four main groups. Most bacteria are decomposers that consume simple carbon compounds, such as root exudates and fresh plant litter, and through this process, they convert the energy stored in soil organic matter into forms useful to the rest of the organisms in the soil food web. A number of decomposers can even break down pesticides and pollutants found in the soil. However, what makes decomposers particularly important is that they immobilize or retain nutrients in their cells, thus preventing the loss of critical elements, such as nitrogen, from the rooting zone of plants (Ingham 2013).

Another of the two groups of soil bacteria are the pathogens and the lithotrophs. The first of these, such as the species Agrobacterium, can cause gall formation in plants, and other

diseases, while the second of these, also known as the chemoautotrophs, obtain their energy from compounds of nitrogen, sulfur, iron or hydrogen instead of from carbon compounds. A few of each of these various types of bacteria play minor roles in nitrogen cycling and the degradation of pollutants (Ingham 2013), but neither the pathogens or the lithotrophs have a significant role in the overall health of the soil ecology.

The last group of bacteria are the mutualists. These bacteria form partnerships with plants, the most well-known of which is the nitrogen-fixing one. Plants get all the nitrogen they need from the soil. Yet the most plentiful source of nitrogen is in the air, and this is where the bacteria come into play. The Nitrogen found in the air becomes a part of biological matter mostly through the actions of bacteria and algae in a process known as nitrogen fixation. Nitrogen fixing bacteria take nitrogen gas from the air and convert it first into ammonium, NH_4^+. Then other bacteria further convert ammonium into nitrite ions, NO_2- and finally into nitrate ions, NO_3^-, which plants can utilize as a nutrient for their growth. In particular, Nitrogen is incorporated into amino acids which plants use to make their proteins, which as enzymes make the chemical reactions take place that run cells. Without the cells of a plant functioning, the plants would eventually die. That, in turn, would lead to the death of the primary consumers since they would no longer have a source of energy, and eventually lead to the death of the secondary consumers since they, too, would be in the same predicament. Hence, without the nitrogen fixing bacteria, eventually the ecosystem would collapse.

Given this significance that bacteria have in the soil, any disruption to the lives of these critical microbes could potentially put an entire ecosystem at risk, and one way that humans are definitely having a positive negative impact on the soil bacteria is how they dispose of their trash. Trash affects the environment in an extremely negative way because when it is put in

landfills, the landfills destroy useful land, and it may take many years before the land regains all of its nutrients back (Lin, 2013). Plastic waste, for example, can sink into the soil where it is disposed (Knoblauch, 2009), and there it can stay for hundreds to even thousands of years before completely degrading (Barnes, Galgani, Thompson, Barlaz, 2013). Likewise, the only way for aluminum (another common trash) to be broken down is for it to be decomposed, which takes about 200-500 years (SPO, 2006). The reason for this is that while soil bacteria can break down the sodium oxalate in alumina, (which is the oxide for aluminum), they are incapable of breaking down the actual heavy metal in the can (Science, 2013).

The sad part is that aluminum is 100% recyclable, and if humans would simply dispose of their aluminum cans in this way, much of the tons of trash that are already metals (8.5% of all trash) (EPA, 2012), would not harm the earth's surfaces. Furthermore, science has identified more than 600 types of bacteria that can biodegrade plastic waste for energy purposes, and humans now use a polymer consumed by these bacteria to produce plastics of all kinds that can biodegrade (The College Street Journal, 1997). Therefore many types of plastic waste can now be disposed of with minimal impact on the environment because of soil bacteria.

However, given that a lot of aluminum and plastic trash are still disposed wastefully, we wanted to see if plastic or aluminum had a greater negative alter on the density on bacteria in the soil. To test our question we decided we were going to place plastic bottles and aluminum cans on different plots of soil and compare it with plots with no soil to test what trash has a greater negative alter. We think that the aluminum trash will have a greater negative impact on the ecosystem and the density of the bacteria living in the soil.

Lab Report

I. Problem: Does plastic or aluminum trash have a greater negative impact on the density of bacteria in the soil.

II. Hypothesis: The aluminum has a greater negative impact on the density of the bacteria in the soil than the plastic does.

III. Procedure:

A. Independent Variable: Type of trash on top of each plot

B. Dependent Variable: Number of bacteria per cubic centimeter of soil

C. Negative Control: plot with no trash on it

D. Controlled Variables:

- What kind of plastic (plastic water bottle)
- What kind of aluminum (soda can)
- Number of days trash is kept on soil (6 days)
- Placement of plots (flat surface)
- Size of plot (40cm x 40cm)
- Amount of plastic put on plots (5 bottles per plot)
- Amount of aluminum put on plots (5 cans per plot)
- Amount of soil sampled (15 centimeters tall by 2.5 centimeters wide)
- Number of plots (9)
- Location of plots
- Size of pipette (10ml)
- Size of culture tube (15ml)
- Type of pipette (serological)

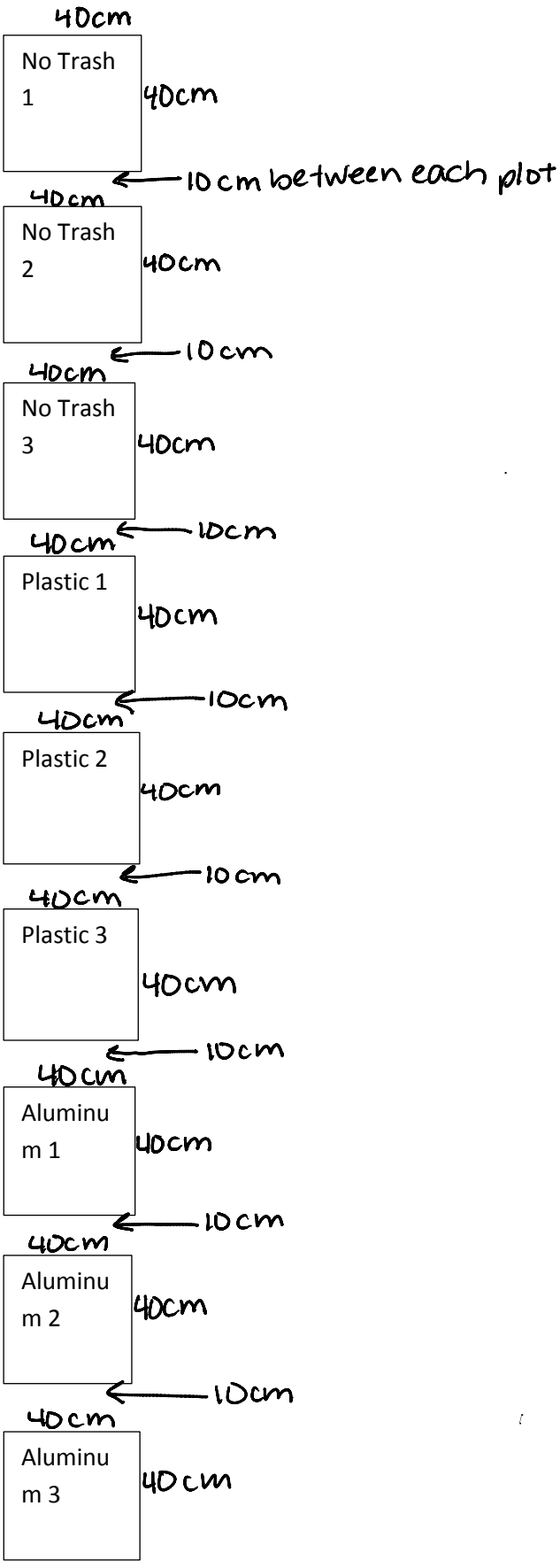
- Type of growing plate
- Amount of soil diluted
- How far about plots are from each other(10cm by 10cm)
- Time allowed for bacteria to grow (72)
- How many levels diluted to (-4)
- Type of plant plots are located on (mustard ivy)
- Number of soil dilution plates (2 per soil)
- Dilutions plated (-3 and -4)
- Amount of dilution put on the plates

E. Step-by-step instructions

1. Go to North: 39.35687 and West: 76.63650 flat ground with flags
2. Use the diagram below to make plots

School Building

BakerRakEvans1



Road

3. Perform steps 4-6 on the same day at the same time.
4. Label 3 bags “No Trash1 Before”. Label each bag A, B, or C. Label 3 bags “Plastic 1 Before”. Label each bag A, B, or C. Label 3 bags “Aluminum 1 Before”. Label each bag A, B, or C. Label 3 bags “No Trash 2 Before”. Label each bag A, B, or C. Label 3 bags “Plastic 2 Before”. Label each bag A, B, or C. Label 3 bags “Aluminum 2 Before”. Label each bag A, B, or C. Label 3 bags “No Trash 3 Before”. Label each bag A, B, or C. Label 3 bags “Plastic 3 Before”. Label each bag A, B, or C. Label 3 bags “Aluminum 3 Before”. Label each bag A, B, or C.
5. Use the soil core sampler to extract 15cm deep by 2cm wide of soil from each of the nine plots and place each sample in its corresponding labeled bag (e.g. “No Trash 1 Before” soil sample in the bag labeled “No Trash 1 Before”)
6. Bring all samples of soil into classroom to dilute soil
7. Perform steps 8-22 on the same day at the same time
8. Use a clean new transfer pipette to add 10ml of sterile waster to a 15ml culture tube. Label tube “No Trash 1A before 10^{0}”
9. Using the same pipette add 9ml to a second 15ml culture tube. Label the tube “No Trash 1A before 10^{-1}”
10. Repeat step 9 three times to three more 15ml culture tubes, only label them “No Trash 1A before 10^{-2}”, “No Trash 1A before 10^{-3}”, and “No Trash 1A before 10^{-4}” respectively.

11. Place 1cc of “No Trash 1A before” soil sample into the “No Trash 1A before 10^{0}” culture tube.
12. Cap the tube and shake vigorously
13. Using a new clean pipette, remove 1ml of the soil/water mixture form the “No Trash 1A before 10^{0}” tube and place it to the “No Trash 1A before 10^{-1}” tube.
14. Cap and shake vigorously
15. Using the same pipette in step 13, remove 1ml of the soil/water mixture form the “No Trash 1A before 10^{-1}” tube and place it into the “No Trash 1A before 10^{-2}” tube.
16. Cap and shake vigorously
17. Using the same pipette in step 13, remove 1ml of the soil/water mixture form the “No Trash 1A before 10^{-2}” tube and place into the “No Trash 1A before10^{-3}” tube.
18. Cap and shake vigorously
19. Using the same pipette in step 13, remove 1ml of the soil/water mixture form the “No Trash 1A before 10^{-3}” tube and place it into the “No Trash 1A before 10^{-4}” tube.
20. There should now be 5 culture tubes.
21. Plate 100µl from the 4^{th} and 5^{th} culture tubes (dilutions “No Trash 1A before 10^{-3}” and “No Trash 1A before10^{-4}”) onto their own separate, corresponding labeled 3M Petrifilm™ aerobic count plates

22. Repeat steps 8-21 with the rest of the soil samples, changing labels to match each sample
23. Allow all plates to grow for 72 hours
24. After 72 hours lay out each individual plate
25. Examine the plate labeled "No Trash 1A before 10^{-4}" first
26. Look for individual bacterial colonies on the plate. If there are less than 5 colonies on the plate labeled "No Trash 1A before 10^{-4}" move on to the plate labeled "No Trash 1A before 10^{-3}"
27. To make your estimates of the bacteria in the original 1cc soil sample using the following formula:

#Microbes in 1cc of soil= #Colonies on sheet x 10^{2} x $10^{|\text{dilution \# at which these colonies were found}|}$

28. Go back to North: 39.35687 and West: 76.63650
29. Place five plastic bottles on the three "plastic plots" and place 5 aluminum cans of the tree 'aluminum plots"
30. Leave plastic and aluminum on plots for 6 days
31. Label 3 bags "No Trash1 After". Label each bag A, B, or C. Label 3 bags "Plastic 1 After". Label each bag A, B, or C. Label 3 bags "Aluminum 1 After". Label each bag A, B, or C. Label 3 bags "No Trash 2 After". Label each bag A, B, or C. Label 3 bags "Plastic 2 After". Label each bag A, B, or C. Label 3 bags "Aluminum 2 After". Label each bag A, B, or C. Label

3 bags “No Trash 3 After”. Label each bag A, B, or C. Label 3 bags “Plastic 3 After”. Label each bag A, B, or C. Label 3 bags “Aluminum 3 After”. Label each bag A, B, or C.

32. Repeat steps 3-27 with new soil samples

IV. Data and Analysis

A. Data Table

Figure 1: The Impact of Trash on Soil Bacteria

		Number of Bacteria in 1cc of No Trash soil		Number of Bacteria in 1cc of Plastic soil		Number of Bacteria in 1cc of Aluminum soil	
Trial	Sample	Before	After	Before	After	Before	After
1	A	7000000	7000000	2600000	3400000	13000000	12000000
1	B	45000000	1400000	2400000	2000000	36000000	2400000
1	C	19000000	9000000	8000000	1100000	2000000	34000000
Average		23666666.67	5800000	4333333.333	2166666.667	17000000	16133333.33
2	A	14000000	700000	2200000	6000000	4300000	10000000
2	B	8000000	1600000	4200000	2200000	800000	6000000
2	C	13000000	2200000	700000	8000000	500000	500000
Average		11666666.67	1500000	2366666.667	5400000	1866666.667	5500000

Figure 2:

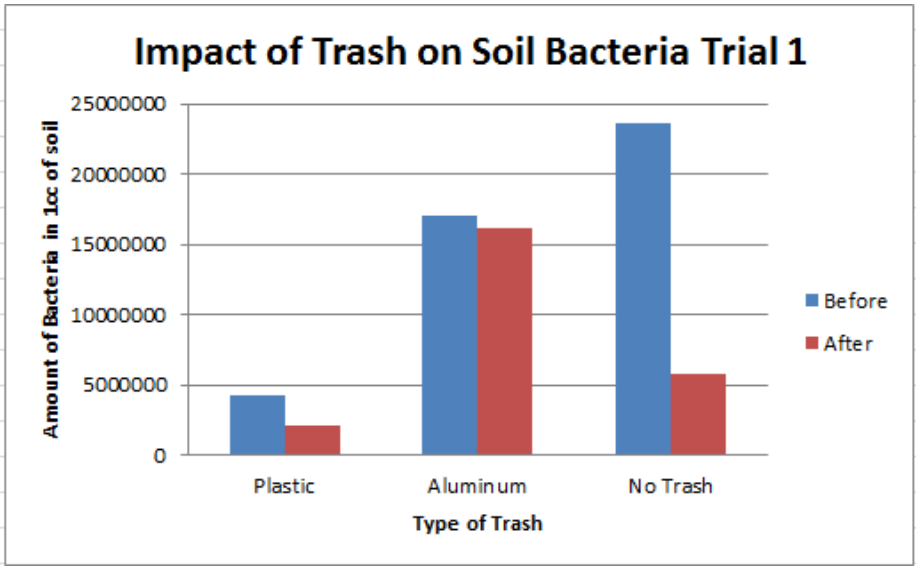


Figure 3:

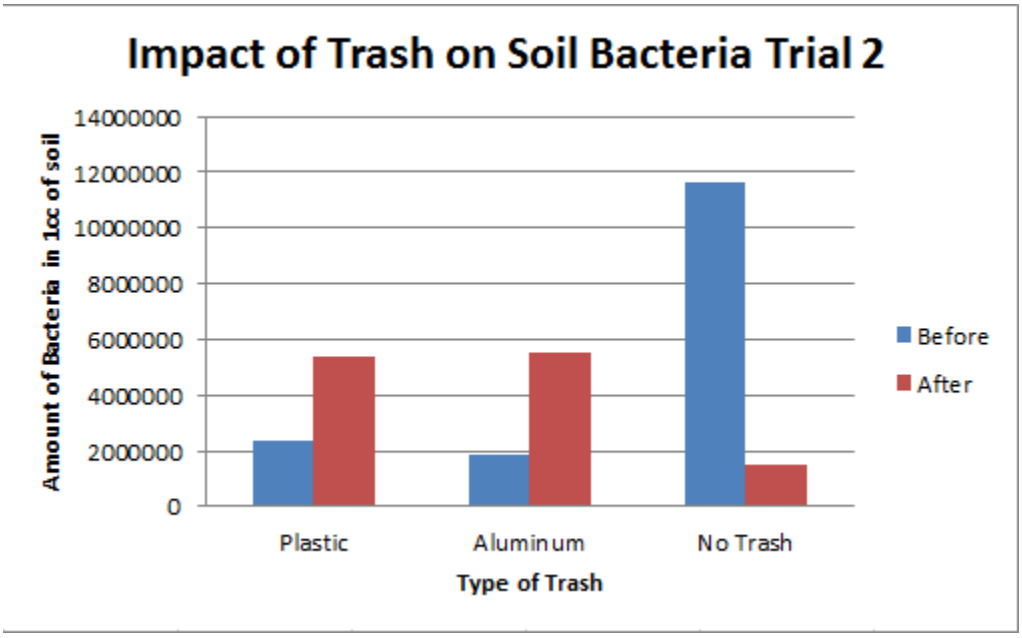

Impact of Trash on Soil Bacteria Trial 2
Amount of Bacteria in 1cc of soil
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
Plastic
Aluminum
No Trash
Type of Trash
Before
After

Figure 4: Percent Change, Corrected Difference, and P Value of soil bacteria

	Plastic 1	Aluminum 1	No Trash	Plastic 2	Aluminum 2	No trash 2
Percent Change	-50%	-5.1%	-75.5%	128.2%	194.6%	-87.1%
Corrected Difference	25.5%	70.4%	0%	215.3%	281.7%	0%
P Value	0.71	0.95	0.25	0.22	0.32	0.03

V. Conclusion

Our hypothesis "the aluminum has a greater negative impact on the density of the bacteria in the soil than the plastic does" was not supported by this experiment. In fact the aluminum had a greater positive impact than the plastic did on the soil bacteria population density. Throughout the experiment regardless of the location of the plots there was an environmental change that had a negative impact on the soil bacteria. This environmental change could have included a heavy downpour of rain which brought too much water to the No Trash plots, and not as much to the plots including trash. This is because the trash could have acted as a barrier to the soil, but whatever the environmental change was it caused a decrease in the density of the soil bacteria. This is demonstrated by the Negative Control plots which included No Trash on top, and shows the drastic decrease in figures 2, 3 and 4. In figures 2 and 3 which include both trial graphs of the before and after number of bacteria present in soil, the number of bacteria present in the No Trash plots went down drastically in both trials. In figure 4 the percent change for trial one was -75.5% and in trial 2 -87.1%, and this data proves the decrease in the density of bacteria soil

population. To furthermore prove this concept of the negative environmental change in figure 4 the p values 0.25 and 0.03 tell us there is a high percent level of certainty that this change did occur.

As seen, the environmental change brought a decrease in the soil bacteria population density. However the plots consisting of trash did not have as much of a decrease. This could be true because the trash on top of the plots blocked the rain from getting into the soil as much as the rain did in the no trash plots. This smaller decrease is shown in figures 2, 3, and 4. In figure 2, by looking at the graph one can tell the number of bacteria for the before and after decrease is smaller in both Aluminum and Plastic plots then in the No Trash plots. However this decrease is backed up more in figure 4 where the percent change of Plastic is -50% and Aluminum -5.1% which is less than the No Trash which is -75.5%. Also by taking away this environmental change by showing the corrected difference in figure 4, the Plastic and Aluminum plots are well above 0 like the No Trash plot which shows it had a positive impact. Overall, in trial 1 Plastic and Aluminum had more of a positive effect on the population density of bacteria in the soil then the No Trash. Also in trial 2, Plastic, Aluminum, and No Trash had the same effect. In trial 2 the No Trash plots have even more of a decrease shown in figure 4, from the percent change going from -75.5% to -87.1%. The percent change in the Aluminum and Plastic plots in trial 2 show even more in a positive effect in figure 4 with the percent change of Plastic being 128.2% and Aluminum being 194.6%. The corrected difference in figure 4 also shows the higher positive effect the Trash plots had vs. the No Trash plot. The Aluminum plot had a corrected difference of 281.7% and the Plastic plot having a corrected difference of 215.3%. Overall in trial 2 the plots containing Trash had more of a positive effect than the plot containing No Trash.

Between the six Trash plots the plots containing Aluminum had more of a positive effect than the Plastic plot. Both plots had a positive affect but based on the data, the aluminum had more of a positive effect. This could be true because while both blocked the rain from soaking the soil too much, the plastic bottles sunk into the soil more than the aluminum cans causing the plastic to have a positive affect yet slightly more negative than the aluminum because of its weight enabling it to sink in more. In figures 2 and 3 by looking at the graphs one can tell aluminum is greater than plastic but looking at figure 4 based on the percentage change in trial 1 plastic had a greater negative percentage change of -50% where aluminum's was -5.1%. Also in trial two in figure 4, aluminum's positive percentage is higher than plastic being 194.6% to 128.2%. Also in looking at figure 4 the corrected difference for both aluminums are greater than the plastics corrected difference of 70.4% and 281.7% to 25.5% and 215.3&. This proves that aluminum had a greater positive effect than plastic.

In conclusion, our hypothesis "the aluminum has a greater negative impact on the density of the bacteria in the soil than the plastic does" was not supported by this experiment. Based on this, we would like to further research if aluminum would have the same positive impact on other soil microbes, such as fungi or protozoa.

Work Cited

Agriinfo. (2011). Soil Microorganism: Bacteria. Prowebs http://agriinfo.in/?page=topic&superid=5&topicid=147

Alejo, J. A. (2008). The Role of Fungi and Bacteria on the Organic Matter Decomposition Process in Streams: Interaction and Relevance in Biofilms. University of Girona.

http://dugi-doc.udg.edu/bitstream/handle/10256/4817/Tjaa1de1.pdf?sequence=1

Atuanya, E. I., Aborisade W. T., Nwogu N. A. (2012) Impact of Plastic Enriched Composting on Soil Structure, Fertility and Growth of Maize Plants. IDOSI Publications.

http://idosi.org/ejas/4(3)12/3.pdf

Barnes, D. K. A., Galgani, F., Thompson, R. C., Barlaz, M. (2013) Accumulation and Fragmentation of Plastic Debris in Global Environments. The Royal Society.

http://www.jstor.org/stable/pdfplus/4640503.pdf?acceptTC=true

The College Street Journal. (1997) Remarkable Bacteria Can Make Biodegrading Plastic and Even Eat Toxic Waste. Mount Holyoke College Office of Communications.

https://www.mtholyoke.edu/offices/comm/csj/970124/bacteria.html

Elmhurst College. Nitrogen Cycle. CHM 110 - CHEMISTRY AND ISSUES IN THE ENVIRONMENT

http://www.elmhurst.edu/~chm/onlcourse/chm110/outlines/nitrogencycle.html

EPA, (2012) Wastes – What You Can Do.

http://www.epa.gov/osw/wycd/catbook/what.htm

Fredrickson, J. K. & Onstott, T. C. (1996) Microbes Deep inside the Earth. Scientific American, Inc.

http://www.nature.com/scientificamerican/journal/v275/n4/pdf/scientificamerican1096-68.pdf

Hoorman, J. J. & Islam, R. (2010) Understanding Soil Microbes and Nutrient Recycling. The Ohio State University.

http://ohioline.osu.edu/sag-fact/pdf/0016.pdf

Ingham, E. R. (2013) Soil Biology. United States Department of Agriculture.

http://soils.usda.gov/sqi/concepts/soil_biology/bacteria.html

Kimball, J. W. (2011). The Nitrogen Cycle. The Saylor Foundation.

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NitrogenCycle.html

Knoblauch, J. A. 2009. The Environment Toll of Plastics. Environmental Health News.

http://www.environmentalhealthnews.org/ehs/news/dangers-of-plastic

Lin, J. (2013) The Effects of Garbage on Our environment.

http://ezinearticles.com/?The-Effects-of-Garbage-on-Our-Environment&id=6556212

Moore, G. T. (1913) Micro-Organisms of the Soil. Scientific American, Inc.

http://www.nature.com/scientificamerican/journal/v75/n1935supp/pdf/scientificamerican 0201 1913-74supp.pdf

National Center for Atmospheric Research. The Nitrogen Cycle

http://www.eo.ucar.edu/kids/green/cycles7.htm

Russell, Allen S. (2012) Aluminum. Hill Global Education Holdings, LLC

http://www.accessscience.com/content.aspx?SearchInputText=aluminum&id=026400

Scharf, R. (1994) Evidence for the interference of aluminum with bacterial porphyrin biosynthesis.

http://www.ncbi.nlm.nih.gov/pubmed/8148615

Science Fresh. (2013) Bacteria munch up alumina impurities. PHYS.

http://phys.org/news198768264.html

SPO (2006) Waste Management & Recycling Program. Maine.

http://www.maine.gov/spo/recycle/mainerecycles/enduringlitter.htm

Travis, J. (1998) Novel bacteria have a taste for aluminum. Science News.

http://www.sciencenews.org/sn_arc98/5_30_98/fob2.htm