

Analysis of Fecal Coliform Levels at Ellejoy Farm

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Abstract: Ellejoy Farm is a piece of property located approximately 13 miles southeast of Knoxville in Blount County. Ellejoy Farm is located within the Little River subwatershed of the Fort Loudon watershed, Hydrologic Unit Code 06010201. Ellejoy Creek, which runs through the property, is listed as problematic under Tennessee's 303(d) category of impaired water bodies in part because of the high levels of fecal coliform bacteria and E. coli found within the creek. Ellejoy Farm is suspected to be a source of nonpoint pollution within the watershed due to the levels of fecal coliform and E. coli found within the creek as it runs through the property. Although Ellejoy Farm was used as pastureland for cattle many years ago, the farm is now used as cropland or left fallow, leading to the supposition that any contributions made to the bacteria levels in Ellejoy Creek from the farmland are the result of residual bacteria that have been sequestered in the soil since the property was pastured. Soil was sampled from areas that were suspected to be contributing and analyzed using the IDEXX Quanti-Tray®/2000 system, and polyacrylamide blends were utilized in an attempt to improve result clarity. Upon examination of the results it was decided that the soil at Ellejoy Farm may be a reservoir for fecal coliform bacteria, but further analysis and refinement of methodology is needed to make conclusions confidently.

Introduction: The use of land as pasture for grazing animals, especially cattle, is a common agricultural practice. Over time the manure from pastured animals accumulates on the soil surface and is incorporated into the soil organic matter. In addition to contributing carbon and other nutrients to the soil, animal manures may also contain

pathogens such as bacteria and other contaminants that can be harmful to humans if they leach into a water source. Fecal coliform bacteria are organisms that may serve as indicators to the presence of other, more harmful contaminants. The land at Ellejoy Farm, the future site for a Tennessee Agricultural Experiment Station research dairy farm in Blount County, includes fields that were pastured for many years. The soil may still have a reservoir of contaminants found in animal wastes. Sampling the soil at different locations on Ellejoy Farm and analyzing the concentrations of fecal coliform was performed in order to provide information on residual contaminants in the soil. This information may provide insight as to contaminant movement off of the fields and out of the soil profile into nearby water systems, potentially contaminating water elsewhere. If there is high residual fecal coliform on the fields then there is a high probability that other contaminants, including pathogens that could cause harmful effects to human health, also reside in the soil and have the potential to move into the water system.

Objectives: To disprove the following null hypotheses:

1. There is no residual fecal coliform in the old pasturelands at Ellejoy.
2. Residual fecal coliform at Ellejoy is not in high enough concentrations to contribute to poor water quality.

The nutrients and organisms found in animal wastes from agricultural lands are considered to be nonpoint sources of pollution in water (USEPA, 1994). Animal wastes such as cattle manure are a major contributor to contaminants in the hydrologic system (Reddy et al., 1981). As there are many types of microorganisms found in animal wastes,

highly abundant indicator organisms such as fecal coliform are used to detect the potential for other contaminants (USEPA, 2001). Fecal coliform bacteria may be carried by runoff into surface waters or become adsorbed to sediment and move through the soil profile into groundwater (Gerba and Bitton, 1984). Contamination of water sources by microbial pathogens may be related to the ability of the pathogens to survive in soil over long periods of time, which can be affected by soil type and land management (Jamieson et al, 2002). Analyzing the presence and concentration of fecal coliform bacteria in the soil at Ellejoy farm after being unpastured for several years will help determine long-term microbial survival in the soils and potential for transport of the microbes into the hydrologic system. Differential survival in different soil types and comparison to similar soil where cattle have not been pastured will provide information for a farm management system.

Materials and Methods: On three sampling dates between January and February, soil samples were collected using a trowel and plastic Ziploc bags at Ellejoy farm in Blount County. The soil was removed from surfaces with little vegetative cover, and only the top three to four inches of soil were removed. Prior to removal of the soil sample, the soil's temperature was taken using a probe sensor stuck into the first several inches of the soil. In between soil samples the trowel was cleaned off using a rag to avoid contamination of the samples. The sample sites were located along the edge of a ditch running through Ellejoy Farm that reportedly has high levels of fecal coliform bacteria when flowing. A sampling site was also chosen on a field near to the ditch to see if the bacteria were coming from off of the field rather than from along the ditch. On the

February 16th sampling date, GPS coordinates of the sampling locations were taken using Dr. Logan's GPS unit. On each sampling date the soil samples were kept cool in an ice chest during transport back to the lab on the University campus. Upon return to the campus, the samples were treated differently on each sampling date in an attempt to find the best method for achieving sample clarity for analysis purposes.

On January 25th, the first day of sampling, the samples were brought back to the lab and then diluted with distilled water. Each sample was removed from the cooler and between 25 and 30 grams of each sample were poured out into separate, clean mason jars. The mason jars were filled halfway with distilled water and shaken in order to disperse the soil samples and detach the microbes of interest similar to detachment that would occur during an intense storm event. The samples were allowed to settle for 30 minutes in order to precipitate the heavier and larger soil particles. After the samples had settled, 100 mL of the water and suspended particles was poured into an IDEXX sampling bottle and a Colilert® packet was added to the sampling bottle. The bottle was capped and shaken in order to dissolve the Colilert®. The bottle was then opened and the contents were poured into an IDEXX Quanti-Tray®/2000, which was placed into a rubber mat designed to fit the tray and run through the IDEXX heat-sealer. The tray was labeled with the site information and put into an incubator set at 37° C. This process was used for each sample. 100 mL of the distilled water that was used was also subjected to the process in order to confirm that the water has pure. All of the IDEXX trays were incubated for 26 hours and removed on January 26th for analysis. The trays were compared to an IDEXX example tray, which displays the minimum color change for a well to be positive. The wells turn yellow to indicate the presence of fecal coliform, and

are fluorescent under a blacklight when *E. coli* is present in a sample. This occurs because the Colilert® contains two nutrients that are specific to fecal coliform and *E. coli*. The nutrients are ONPG (o-nitrophenyl- β -D-galactopyranoside), which is metabolized by fecal coliform bacteria using the enzyme β -galactosidase, and MUG (4-methylumbelliferyl- β -D-glucuronide), which is metabolized by *E. coli* using the enzyme β -glucuronidase. The indicator nutrient ONPG turns yellow after metabolism due to hydrolysis and release of o-nitrophenol, and the MUG becomes fluorescent after metabolism due to hydrolysis and the release of 4-methyl-umbelliferone. The number of positive large wells and positive small wells for both fecal coliform and *E. coli* was used in order to determine the number of colony forming units in each 100 mL sample using the MPN, or most probable number, method. This method allows for 95% accuracy on counts up to 2,419.6 colony forming units per 100 mL (IDEXX, 2008). Unfortunately, due to the presence of suspended clay particles in the samples assessed, the clarity of results was not up to the desired standards. Rather than allow the samples to settle longer after being shaken in the mason jar, which would also allow microbes to settle, an alternative method was attempted the following week using soils with known high levels of fecal coliform bacteria.

On the February 1st sampling date, conditions were too rainy for driving on the unpaved roads at Ellejoy Farm. Soil samples were instead taken from the current University of Tennessee dairy farm in a field that has had cattle pastured on it recently. Sampling sites with poor vegetation cover were chosen for this site also and the samples were taken in the same manner as at Ellejoy. Samples were also taken from the University campus, where there should be much lower levels of fecal coliform due to a

lesser amount of animal wastes. A portion of each sample taken was added to separate, clean mason jars, mixed with deionized water, and set aside to see how long settling would take. Polyacrylamide emulsions from the APS 600 series from Applied Polymer Systems, Inc. were borrowed from Dr. Buchanan in the wastewater treatment lab.

Polyacrylamides are a relatively new technology used as a best management practice for controlling erosion and improving the water quality of stormwater runoff from sites with bare soil. A linear, anionic polyacrylamide emulsion is used to flocculate the soil particles, including the smaller particle sizes, and cause them to precipitate more quickly. The polyacrylamide flocculates soil particles using coulombic and Van der Waals forces, which can be different depending on the composition of the soil (Sojka et al, 1998). For the soil tests in this experiment, the APS 605 emulsion was used. Because it was unsure which proportions of the emulsion to use to best remove the sediment without also removing the microbes, each dairy farm sample was treated differently. Each sample had approximately between 5 and 10 grams of soil added to 8 ounces of deionized water in a clean mason jar, and the samples were shaken thoroughly. Once the larger soil particles had settled, the murky water was transferred to another clean mason jar, with care taken not to disturb the larger soil particles at the bottom of the container. The first dairy farm sample received 1 mL of straight emulsion. For the second dairy farm sample, 1 mL of the emulsion was mixed with 50 mL of deionized water before being added to the water sample. For the third dairy farm sample, 1 mL of the emulsion was mixed with 125 mL of deionized water before addition to the sample. These steps were taken with each of the three campus samples as well (campus sample 1 being treated as dairy farm 1, etc). The samples were all swirled gently in order to spread the PAM and allow the soil

particles to flocculate. The polyacrylamide solution did not work as quickly as intended, and so the samples were allowed to sit for approximately 2 hours to completely flocculate and settle. After settling, 100 mL of each sample was poured into the IDEXX sample bottles, mixed with Colilert®, sealed, and incubated at 37°C for 24 hours before they were removed and analyzed. At that time, the samples that had been set aside were checked to see if they had experienced sufficient settling, which was not the case. Because of this, the samples were once again mixed with deionized water, allowed to settle, and poured off into clean mason jars so that different quantities of emulsion and deionized water were mixed and added to the samples to determine the best mixture for settling out the soil as quickly as possible. Mixtures that were tried included 375 mL deionized water with 3 mL of emulsion, 250 mL of water and 2 mL of emulsion, 200 mL of water and 1 mL of emulsion. These solutions were then diluted further until it was determined that the most effective mix of emulsion and deionized water was 120 mL of water and 0.4 mL of emulsion. This was the emulsion consistency used for the soil samples for the next two sampling dates.

On the February 8th sampling date more samples were taken from the sites at Ellejoy following the standard procedure, and different amounts of soil, water, and emulsion stock solution were tried. For the field sample, approximately five grams of soil was added to 2 ounces of water in a clean mason jar, shaken, and allowed to settle. The water was poured off into another clean mason jar, and the emulsion stock solution was added in quantities of 0.1 mL and swirled until the soil flocculated, which came to 0.6 mL of stock solution. For the first ditch sample, approximately 2 ounces of soil was mixed with 2 ounces of DI water, allowed to settle, and the emulsion stock solution

addition process was repeated up to 0.4 mL. For the second ditch sample, approximately 15 grams of soil was added to 4 ounces of water, allowed to settle, poured off into a new mason jar, and the stock solution was added in 0.1 mL quantities until 3 mL had been added. For the third ditch sample, approximately five grams of soil was added to 2 ounces of water in a clean mason jar, shaken, and allowed to settle. The water was poured off into another clean mason jar, and the emulsion stock solution was added in quantities of 0.1 mL and swirled until the soil flocculated, which came to 0.8 mL of stock solution. The fourth ditch sample received the same treatment as the first ditch sample, including the addition of 0.4 mL of stock solution. The same quantities were used for the fifth ditch sample, but that sample required 0.8 mL of stock for the soil to flocculate. The clear water from each was poured off into an IDEXX sample bottle, amended to 100 mL with deionized water, shaken with Colilert®, run through the IDEXX Quanti-Tray®/2000 process, and incubated at 37°C for 28 hours.

On the February 15th sampling date, the samples were taken from Ellejoy using the standard procedures and transported back to the lab. For the first and second ditch samples, 5 grams of soil were added to separate clean mason jars, amended with 4 ounces of deionized water, shaken, allowed to settle, and the water poured off into a new clean mason jar. The stock solution was added up to 0.5 mL for each. The other samples were treated the same but with 10 grams of soil each, and the quantities of stock solution required for each one were different. The third ditch sample required only 0.1 mL of stock solution. The fourth ditch sample received 0.6 mL of stock solution, the fifth required 0.5 mL, and the field sample only required 0.1 mL of stock solution. As with before, the clear water from each mason jar was poured into the IDEXX sample bottles,

amended to 100 mL with the deionized water, treated with Colilert®, and sealed. The samples were incubated at 37°C for 28 hours.

Results and Discussion: The data shows that all of the soil samples had relatively high concentrations of fecal coliform bacteria, reaching the maximum detection limit of the IDEXX system for the majority of the samples. Alternately, the levels of E. coli found in the soil samples were relatively low, with the exception of the PAM testing sites at the current research dairy farm. The samples taken the same day from the sites on the agricultural campus were unclear, and so the results could not be effectively compared. After going over the data, it appears that the polyacrylamide may have interfered with the fecal coliform and E. coli results, as the wells fluorescence was extremely difficult to detect, which may have led to false negatives concerning the E. coli levels. Additionally, it appeared that the counts for fecal coliform bacteria were higher using the polyacrylamide method of settling than with simply shaking the soil samples with water and allowing the larger particles to settle prior to analysis. This may confirm that the polyacrylamide interfered with the readings, as the amount of soil used in the PAM testing was much lower than the amount of soil used when simply diluting with water. The original method of dilution with water may have led to false positives or false negatives when counting the fecal coliform levels: the turbidity of the water, including suspended clay particles, may have caused wells to appear yellow even without the presence of fecal coliform. Alternately, the microbes may have remained attached to the soil and not been counted in the analysis. Found on the following pages are tables displaying the levels of fecal coliform and E. coli found by the site from which the

samples were taken, as well as the temperature data and relative clarity of the trays from which the data was obtained. Also included is a satellite image of the sampling sites on Ellejey Farm and the location of the farm relative to Knoxville (fig. 1 and 2, respectively).

Ditch 1	temperature (°F)	incubation period	fecal coliform (cfu/100mL)	E. coli (cfu/100 mL)	unclear	coordinates
1/26/2008	40.8	26 hrs	148.3	1	*	N35 45.976 W 83 50.920
2/9/2008	55.9	28 hrs	2419.6	4.1	*	
2/16/2008	53.9	28 hrs	579.4	1		

Ditch 2	temperature (°F)	incubation period	fecal coliform (cfu/100mL)	E. coli (cfu/100 mL)	unclear	coordinates
1/26/2008	41.1	26 hrs	2419.6	9.7	*	N35 45.988 W083 50.958
2/9/2008	53.9	28 hrs	2419.6	1	*	
2/16/2008	54.6	28 hrs	2419.6	1	*	

Ditch 3	temperature (°F)	incubation period	fecal coliform (cfu/100mL)	E. coli (cfu/100 mL)	unclear	coordinates
1/26/2008	42.4	26 hrs	1413.6	6	*	N35 46.040 W083 51.024
2/9/2008	50.3	28 hrs	2419.6	1	*	
2/16/2008	55.7	28 hrs	2419.6	1		

Ditch 4	temperature (°F)	incubation period	fecal coliform (cfu/100mL)	E. coli (cfu/100 mL)	unclear	coordinates
1/26/2008	40.8	26 hrs	613.1	4	*	N35 46.064 W083 51.013
2/9/2008	63.3	28 hrs	2419.6	1		
2/16/2008	56.8	28 hrs	2419.6	14.6		

	temperature (°F)	incubation period	fecal coliform (cfu/100mL)	E. coli (cfu/100 mL)	unclear	coordinates
Ditch 5						
1/26/2008	40.6	26 hrs	261.3	2	*	N35 46.103 W083 51.031
2/9/2008	58.6	28 hrs	2419.6	1	*	
2/16/2008	56.3	28 hrs	2419.6	3.1	*	

	temperature (°F)	incubation period	fecal coliform (cfu/100mL)	E. coli (cfu/100 mL)	unclear	coordinates
Ditch 6						
1/26/2008	41.5	26 hrs	2419.6	4.1	*	N35 46.183 W 83 51.147
2/9/2008			flooded	flooded		
2/16/2008			flooded	flooded		

	temperature (°F)	incubation period	fecal coliform (cfu/100mL)	E. coli (cfu/100 mL)	unclear	coordinates
Field						
1/26/2008	39	26 hrs	17.7	5.2	*	N35 45.989 W 83 50.970
2/9/2008	56.3	28 hrs	2419.6	30.9		
2/16/2008	62.2	28 hrs	2419.6	1		

PAM trials

site	temperature	e.coli (total)	fecal coliform (total)
dairy farm 1	51.3	2419.6	2419.6
dairy farm 2	51	227.7	2419.6
dairy farm 3	48	152.9	2419.6
campus 1	46.5	-	-
campus 2	47.6	-	-
campus 3	47.8	-	-



Figure 1



Figure 2

Conclusions: Although there is great need for further assessment of microbial levels in the soils at Ellejoo, the results found may indicate a deeper problem. Microbial survival in soil is dependent on several factors, including climate, pH, the die-off rate of the microorganism, temperature, and soil particle distribution (Reddy et al, 1981). One of the main factors that could affect the survival of microorganisms at Ellejoo is soil moisture, with microbial survival increasing with increasing soil moisture. The soils at Ellejoo near the sampling sites were often saturated upon observation, even when there had not been a storm even for several days. This could contribute to the high levels of fecal coliform found on the landscape. Also, several game trails were observed near the areas sampled along the ditch at Ellejoo. Although this may not explain the high level of fecal coliform in the field, fecal deposition from wildlife could be the source of fecal

coliform bacteria rather than sequestration in the soil matrix. The results at this time are inconclusive, partially due to the difficulty in obtaining standardized data. The reason for using the IDEXX Quanti-Tray®/2000 method of analysis for fecal coliform and E. coli levels in water is that the system is simple and easy to use. When attempting to use the system in order to analyze soil samples for the same microbial information, it was found that the analysis was difficult due to the need for water clarity in order to properly read the results. While the use of a polyacrylamide emulsion to flocculate and remove the soil particles once a soil sample had been mixed with water helped with the water clarity and appeared to leave the microbes suspended, the results, especially concerning E. coli levels, sometimes became harder to read. In the future, should such an experiment be repeated, there are several steps that should be taken before attempting to find any usable data. First off, should the polyacrylamide method of soil separation be used to flocculate the soil particles and produce a clear water sample to be run through the IDEXX system, the appropriate mixture of emulsion base and deionized water should be found for the soils in question. While in this experiment the APS 605 emulsion was used, different emulsions work on different soils, and a number of emulsions should be tested to find which one works the most quickly and effectively on the soils. The reason for this is that the efficacy of polyacrylamide polymers is based on the texture, salinity, and structure of the soil (Wu, 2001). Once the correct emulsion mixture is found, an attempt should be made to standardize the amount of soil and water obtained from each sample to be mixed with the polyacrylamide, so that the colony forming units per 100 mL can be correlated with a specific volume of soil. As soon as the volume and emulsion standards are found, the soils can be sampled, diluted, and separated with the polyacrylamide and run through

the IDEXX Quanti-Tray®/2000 process, but a portion of the soil samples should also be set aside and analyzed using the standard methods for finding microbial concentrations in soil. By doing this, the results from the IDEXX samples and the results from the standard methods can be compared on a colony forming unit per volume soil basis to ensure that the results from the soil separated with polyacrylamide are accurate, or at least in order to find a standard disparity from which the actual values can be calculated.

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