Fecal Coliform Distribution in Water at Ellejoy Farm

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Abstract: Pathogens, both viruses and bacteria, are of importance to human health because of the negative effects caused by the organisms. The primary way humans are impacted by pathogens is through contact with some form of biological waste. Animal wastes contain various forms of pathogens and nutrients that can be beneficial but are often detrimental when applied to the land; these nutrients and microbes are leached or washed from the surface and transported into the hydrologic system through runoff and subsurface movement of water. The higher the concentrations of pathogens in animal wastes that are land applied or simply deposited on the surface the higher the concentrations of pathogens in the runoff, streams, and potentially the groundwater aquifers as well. Areas that have been farmed are often of concern in terms of pathogen deposition and transport. Ellejoy is the new site for the University of Tennessee research dairy farm located in Blount County. This farm is of particular interest because initial sampling showed that the water and the surrounding soil had high levels of fecal coliform even though livestock have not been pastured there for many years. Sampling the stream system above and below where Ellejoy is contributing to the watershed using the IDEXX system was done to determine if Ellejoy is contributing pathogens and to what degree it is contributing to the overall concentration of the stream's microbial community. At two different points in the stream as it runs across the Ellejoy property the stream sediment was sampled to determine if there is a significant amount of deposition of fecal coliform attached to the soil particles. The concentrations of the pathogens was also determined to see if they are at unsafe levels and this measurement lends a means of assessment to the feasibility of extended use of this land as a livestock farm. High concentrations of fecal

coliform may indicate that the land is probably sensitive to further use for livestock and low concentrations may indicate that the land would be suitable for the continued use of pasturing livestock. This information could lend insight into land management elsewhere with similar soils and watersheds in terms of water quality for future agricultural practices. As the levels of fecal coliform are high it may also be necessary to adjust land or cattle management practices at the site for Ellejoy farm.

Objectives: To disprove the following null hypotheses:

1. There is no residual fecal coliform in the stream sediment at Ellejoy.
2. The amount of fecal coliform in the stream sediment is not related to sediment depth.

Justification: The Clean Water Act was established in the U.S. in 1972 with the intentions to help "restore and maintain the chemical, physical and biological integrity of the Nation's waters" (USEPA, 1972). One factor that can be detrimental to the integrity of a water system is the introduction of pathogens such as fecal coliform bacteria, which are abundant in animal wastes (Kress and Gifford, 1984). Animal wastes are considered to be a non-point source of pollution that may affect groundwater as well as surface waters (O'Connor, 2002). While the land at the proposed site for the dairy farm has not been used for livestock in many years, the likelihood of pathogen sequestration in the soil and subsequent transfer into the water system through runoff and subsurface flow may still contribute to harmful levels of contaminants, especially surrounding storm events (USEPA, 1994). Addition of contaminants that would occur subsequent to reintroduction of large numbers of livestock on the land would exacerbate what appears to be an

undesirable situation. The research was conducted in order to determine whether the pathogens are at a level that is low enough to justify utilization of the land as a dairy farm without concern over contamination of the water source that runs across the property.

Materials and methods: Ellejoy, a farm stream, and a farm ditch were all grab sampled at six different points as the waterways run through the property. Temperature readings were also taken at each of these sites every sampling date. This data was taken to try and identify any trends that would correlate fecal coliform and E. coli concentrations with temperature. These samples of one-hundred milliliters were then assessed for water quality in terms of fecal coliform concentration and E. coli concentrations, especially after storm events to check for increased concentrations. Samples of the stream sediment were taken above the farm to get a base reading of the concentration of fecal coliform in the water before it reaches the Ellejoy farm. Two samples were taken to obtain this base reading; one sample was taken from the farm stream before it entered Ellejoy and then one sample was taken from Ellejoy directly downstream from the point where the farm stream enters Ellejoy. Two samples were needed to obtain the base reading because by comparing the two samples the farm stream's contributions to the fecal coliform and E. coli concentrations can be determined. These samples were then compared with samples of the stream sediment taken below the Ellejoy farm to indicate the amount of fecal coliform and E. coli coming off the land at Ellejoy. Stream sediment samples were also taken within Ellejoy farm to asses if deposition of sediment and attached fecal coliform and E. coli is occurring. The IDEXX system was used in order to determine the levels of fecal coliform and E. coli in the

sediment samples. The IDEXX system is an extremely useful and easily used system for quantifying bacteria in water samples. The IDEXX system requires the volume of the sample to be one-hundred milliliters. At each site two samples were taken; one sample was taken without disturbing the sediment on the bottom of the stream and then another sample was taken after the sediment was suspended in the water by simply disturbing the sediment on the bottom of the stream. These two samples then gave a comparison of the fecal coliform and E. coli living in the flowing water and the fecal coliform attached to the sediment. The samples were taken back to the lab the day they were extracted and incubated with a reagent for at least 24 hours; this reagent called Colilert acts as a marker or indicator for fecal coliform and E. coli and also provides adequate nutrition for bacterial growth during the incubation period. Each sample was brought back to lab, mixed with the reagent and then poured into the Quanti-Tray, which consists of 49 large wells and 48 small wells. These wells are what gives the needed information to use the Most Probable Number Table. The wells that show a positive result are counted and then by looking on the Most Probable Number Table the number of colony forming units per 100 milliliters can be found. These Quanti-Trays were then to be sealed by using the Quanti-Tray Sealer. The Quanti-Trays were incubated from 24 to 28 hours in an incubator at 37 degrees Celsius. After incubation the reagent indicates the presence of fecal coliform by changing the color of the water in each cell containing fecal coliform to a yellow color. Once the count of positive wells was completed the Quanti-Tray was placed under a UV light source and the reagent again indicated the presence of E. coli. The presence of E. coli is determined by the fluorescent glow given off by each well that contain E. coli. As mentioned, a count of colony forming units is accomplished through

the automatic dilution of samples and a count of positive or negative wells on the Quanti-Tray used for incubation (IDEXX, 2007). Blank Quanti-Trays with only distilled water were occasionally run through the IDEXX procedure to ensure there was no contamination and the techniques being used were precise. Once the data had been collected trends were looked for and signs of deposition or flushing of fecal coliform in the stream was analyzed. This analysis will allow for an overall assessment to be done for the continued use of the land for pasturing livestock.

Results: As mentioned, the data was analyzed for any trends. The major trend looked for during the analysis was the correlation of increased concentrations of both fecal coliform and E. coli with the act of disturbing the sediment on the stream bed. This trend was the most important because the purpose of this research was to find any indication that either fecal coliform or E. coli could be attached to the sediment or living in the sediment bed and not only occupying the free flowing water. Other trends that were looked for included the correlation of increased concentrations of fecal coliform and E. coli with increasing temperature, determining if deposition or detachment of fecal coliform or E. coli was occurring in the streams within the property, and trying to determine the impacts of both the farm stream and the farm ditch have on the concentrations of fecal coliform and E. coli in the Ellejoy stream.

The first site that will be discussed is the farm stream. The data is included below where the farm stream is abbreviated as FS and A stands for the sample taken before disturbing the streambed sediment and B stands for the sample taken after disturbing the streambed sediment. The data shows a clear variance of total fecal coliform colony forming units and E. coli colony forming units from each sampling date. Also it is clear

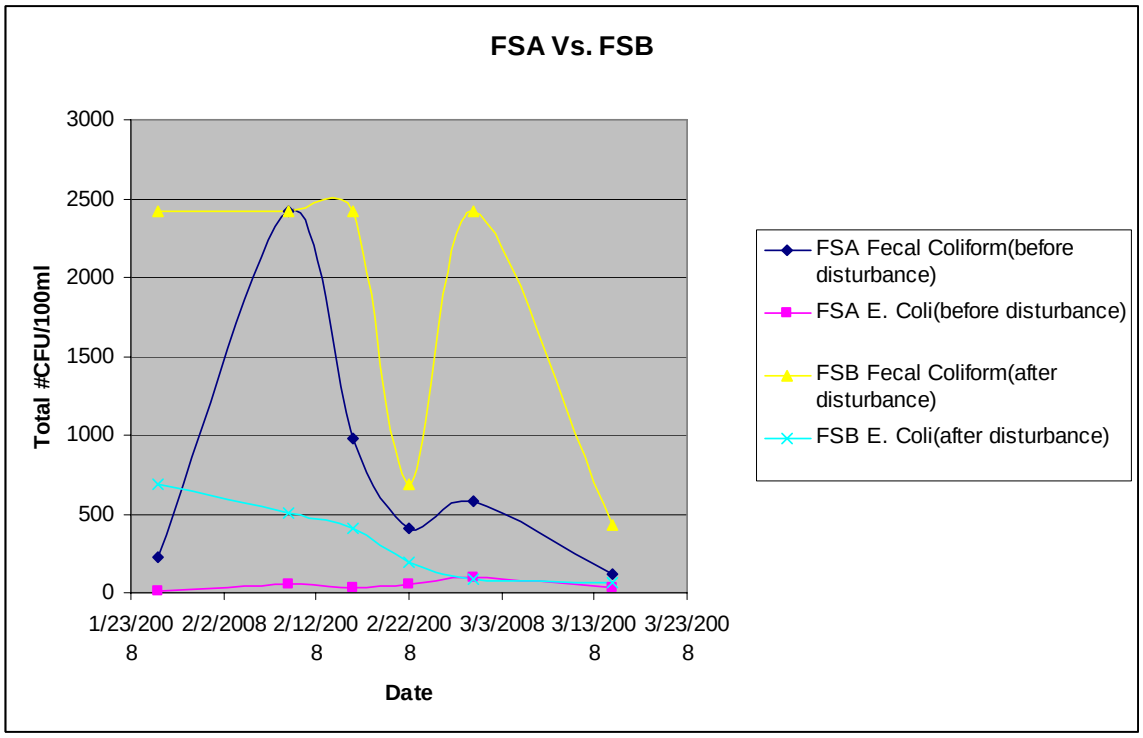
from this site that disturbing the stream sediment on the streambed affects the total colony forming units for both fecal coliform and E. coli. The difference between the samples taken before disturbing the streambed sediment and after disturbing the streambed sediment also varies with sampling dates but in general there is significant increase in the total colony forming units for both fecal coliform and E. coli. However, the degree of increase seems to be much higher for E. coli than fecal coliform which seems to indicate that E. coli inhabits the streambed sediment to greater degree than fecal coliform. The correlation between temperature and total colony forming units of fecal coliform and E. coli could not be identified from this site. In general there does seem to be some relationship between the two because most of the data from this site shows that on warmer sampling days there were higher concentrations of fecal coliform and E. coli in the water. However on some of the cooler sampling days the concentrations are much higher than on a sampling day that was warmer; this may have been caused by a recent storm event prior to the sampling on these cooler days. Storms can cause an increase in fecal coliform and E. coli concentrations when there is a significant amount of runoff produced from that particular storm event. This runoff runs over the surface of the farm picking up sediment and colonies of fecal coliform and E. coli depositing them into the streams that surround this property. When comparing this data with that taken from the first sampling spot on Ellejoy, the contributions of fecal coliform and E. coli from the farm stream cannot be seen. In general the concentrations of fecal coliform and E. coli are both higher in Ellejoy which may indicate that the farm stream is contributing significantly to the concentrations of fecal coliform and E. coli in Ellejoy.

FSA

Date	Temp.	Total fecal Col.	Total E. Coli
1/26/2008	42.4	224.7	7.4
2/9/2008	56.3	2419.6	56.3
2/16/2008	53.6	980.4	28.1
2/22/2008		410.6	58.6
2/29/2008	44	575.6	92.8
3/15/2008	50.3	121	32.2

FSB

Date	Temp.	Total fecal Col.	Total E. Coli
1/26/2008	42.4	2419.6	686.7
2/9/2008	56.3	2419.7	501.2
2/16/2008	53.6	2419.6	408.3
2/22/2008		691	190.4
2/29/2008	44	2419.6	87
3/15/2008	50.3	428.4	64.6



Ellejoy stream itself was sampled in three locations. The first of these sites was directly downstream from where the farm stream emptied into Ellejoy stream. This site is abbreviated as EJ1 and the A and B mean the same as they did in the first data set. The major trend that is the focus of this research is clearly represented by this data. On every sampling date the sample taken after disturbing the streambed sediment contained a higher concentration of fecal coliform and E. coli. Also, this data also supports what the data showed from the farm stream; this data seems to indicate that E. coli inhabits the

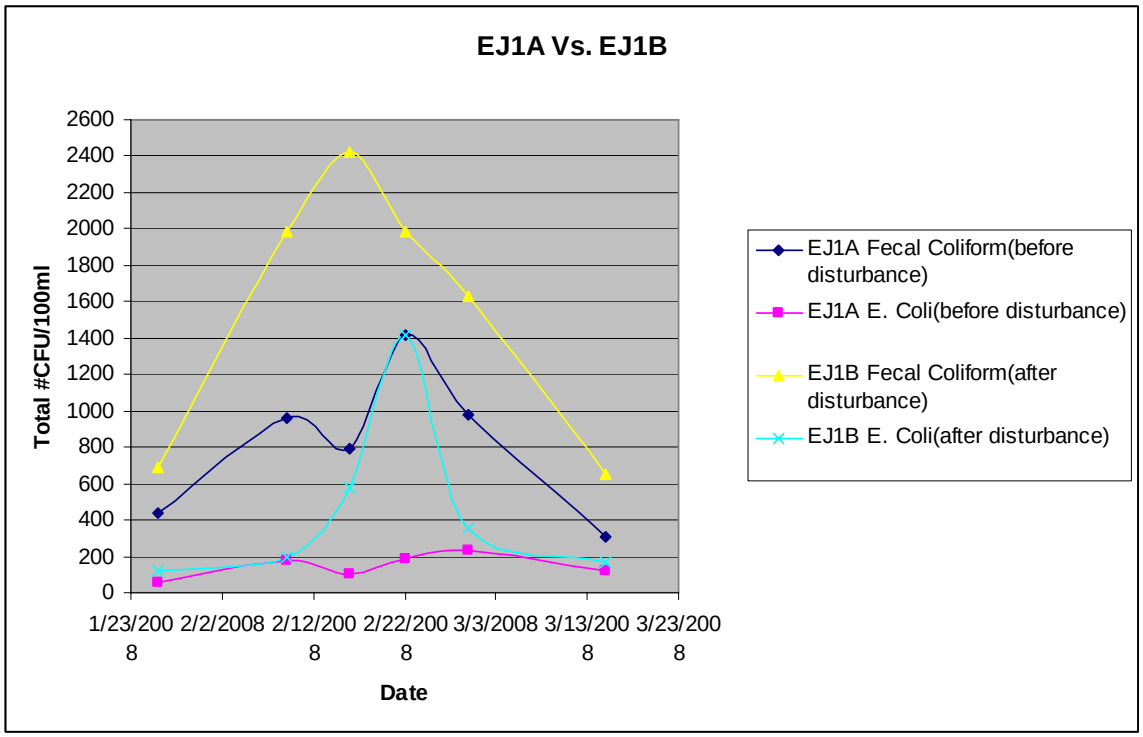
streambed sediment to a greater degree than fecal coliform because the degree of increase in the concentration between samples taken before disturbing and after disturbing the streambed sediment are greater in magnitude for E. coli. Here again the data does not show a clear correlation between temperature and concentrations of fecal coliform and E. coli. However, there is a general trend of increasing concentrations with increasing temperature. This is not always the case but as mentioned the higher concentrations on cooler sampling days could be the result of a recent storm prior to the sampling date. When comparing the data from this site with the data from the site further downstream on Ellejoy there seems to be a trend of detachment of sediment which is carrying fecal coliform and E. coli because most of the time the concentrations of fecal coliform and E. coli were higher at this second sampling site on Ellejoy.

EJ1A

Date	Temp.	Total fecal Col.	Total E. Coli
1/26/2008	39.9	435.2	60.2
2/9/2008	53.9	960.6	179.3
2/16/2008	52.3	791.5	103.9
2/22/2008		1413.6	186
2/29/2008	43.5	976.8	235.6
3/15/2008	52.1	303	124

EJ1B

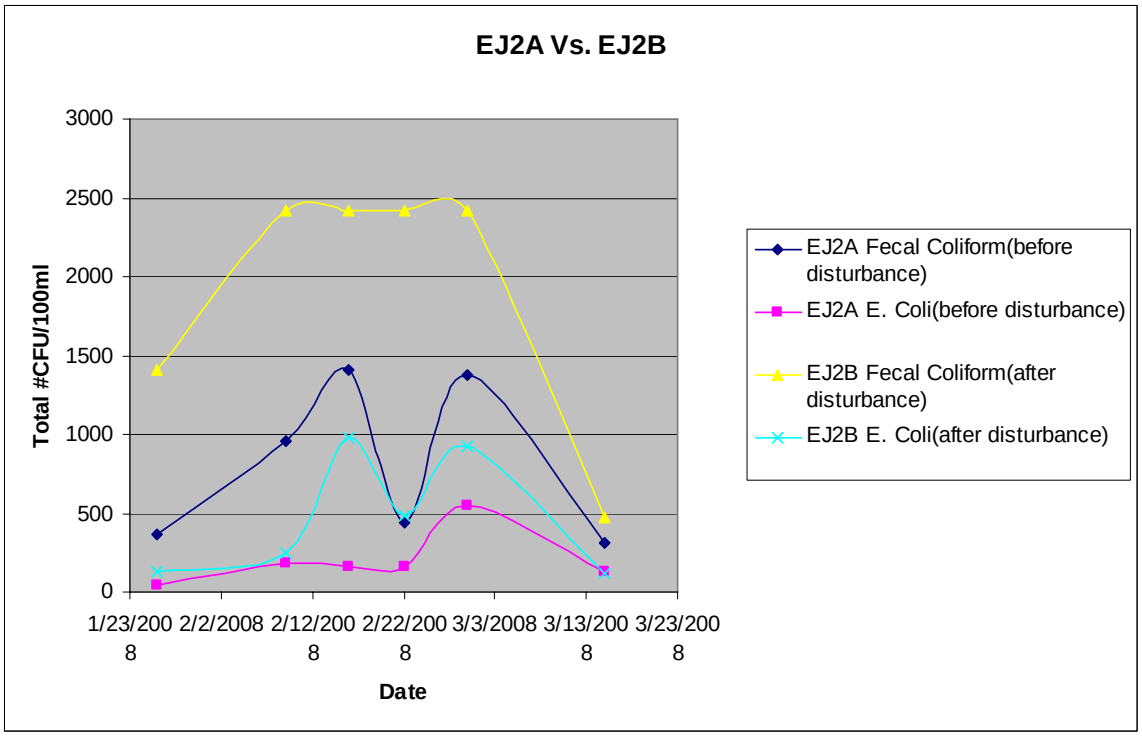
Date	Temp.	Total fecal Col.	Total E. Coli
1/26/2008	39.9	686.7	116.9
2/9/2008	53.9	1986.3	193.5
2/16/2008	52.3	2419.6	579.4
2/22/2008		1986.3	1413.6
2/29/2008	43.5	1632.8	357.8
3/15/2008	52.1	651	167.8



The second sampling site on Ellejoy is abbreviated as EJ2 and as before the A and B stand for the same thing. The data shows for this site that the streambed sediment is harboring colonies of fecal coliform and E. coli. The degree of increasing concentration after disturbance is far greater for fecal coliform than E. coli at this site. This differs from the previous two sites where E. coli showed a greater affinity for the streambed sediment. There could be an explanation for this, and it would be streambed sediment depth. In both the farm stream and at the first Ellejoy sampling spot the streambed sediment was deeper and this may make a difference in the concentrations of E. coli found in the samples after disturbing the streambed sediment. The correlation between temperature and fecal coliform and E. coli concentrations could again not be identified from this data set. The data from this site shows an even smaller degree of correlation than the previous two sites. When comparing the data from this site with the data taken further downstream from the third sampling site on Ellejoy shortly before it leaves the property there seems to be some

indication of deposition throughout this section of Ellejoy stream. This seems to be the case because the concentrations of fecal coliform and E. coli are lower at the third sampling site on Ellejoy stream than at the second sampling site.

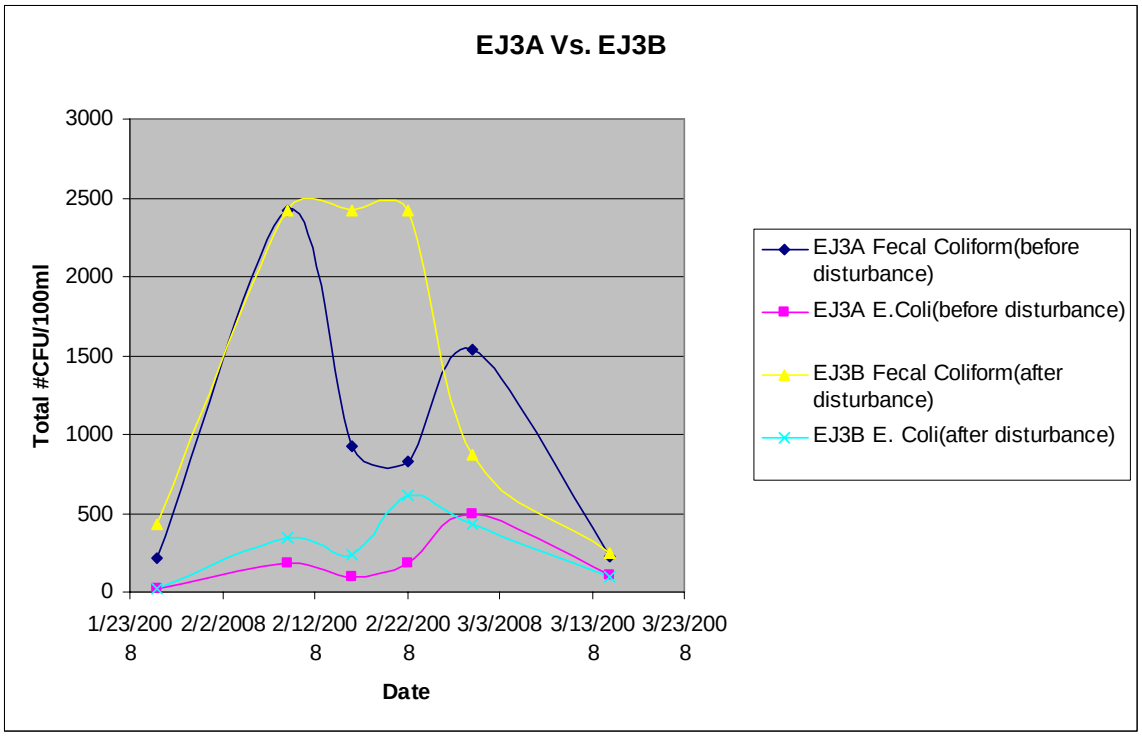
EJ2A				EJ2B			
Date	Temp.	Total fecal Col.	Total E. Coli	Date	Temp.	Total fecal Col.	Total E. Coli
1/26/2008	39.2	365.4	39.3	1/26/2008	39.2	1413.6	129.6
2/9/2008	53	960.6	185	2/9/2008	53	2419.7	248.1
2/16/2008	52.1	1413.6	159.7	2/16/2008	52.1	2419.6	980.4
2/22/2008		437.1	156.5	2/22/2008		2419.6	488.4
2/29/2008	43.7	1373.4	551	2/29/2008	43.7	2419.6	922.2
3/15/2008	51.4	313	126.2	3/15/2008	51.4	471.8	118.2



The third and final sampling site on Ellejoy stream is abbreviated EJ3 and the A and B stand for the same thing as with the previous sites. This data set shows the same major trend; increased concentrations of fecal coliform and E. coli after disturbing the stream bed sediment. However, as at the second sampling site on Ellejoy stream the degree of increased concentration is greater for fecal coliform than for E. coli. This could again be caused by the shallow depth of the streambed sediment. Once again the

correlation between temperature and concentrations of fecal coliform and E. coli can not be identified from this data set. The correlation that seemed to be represented by the first two data sites is not existent here.

EJ3A				EJ3B			
Date	Temp.	Total fecal Col.	Total E. Coli	Date	Temp.	Total fecal Col.	Total E. Coli
1/26/2008		209.8	20.3	1/26/2008		435.2	19.7
2/9/2008		2419.7	185	2/9/2008		2419.7	344.8
2/16/2008	52.2	920.8	93.2	2/16/2008	52.2	2419.6	238.2
2/22/2008		829.7	185	2/22/2008		2419.6	613.1
2/29/2008	42	1540.2	496.2	2/29/2008	42	872	428.6
3/15/2008	50.2	229	102.4	3/15/2008	50.2	246.8	96



The next two sampling sites to be discussed were from the farm ditch. This ditch runs from the middle of Ellejoy farm between several fields and then empties into Ellejoy past the property boundaries. The farm ditch always had water flowing through it at the first sampling site, but the ditch was for the most part dry at the second sampling site. This was not the case after storm events prior to several sampling dates; these storms provided enough runoff volume for the farm ditch to flow with water from beginning to

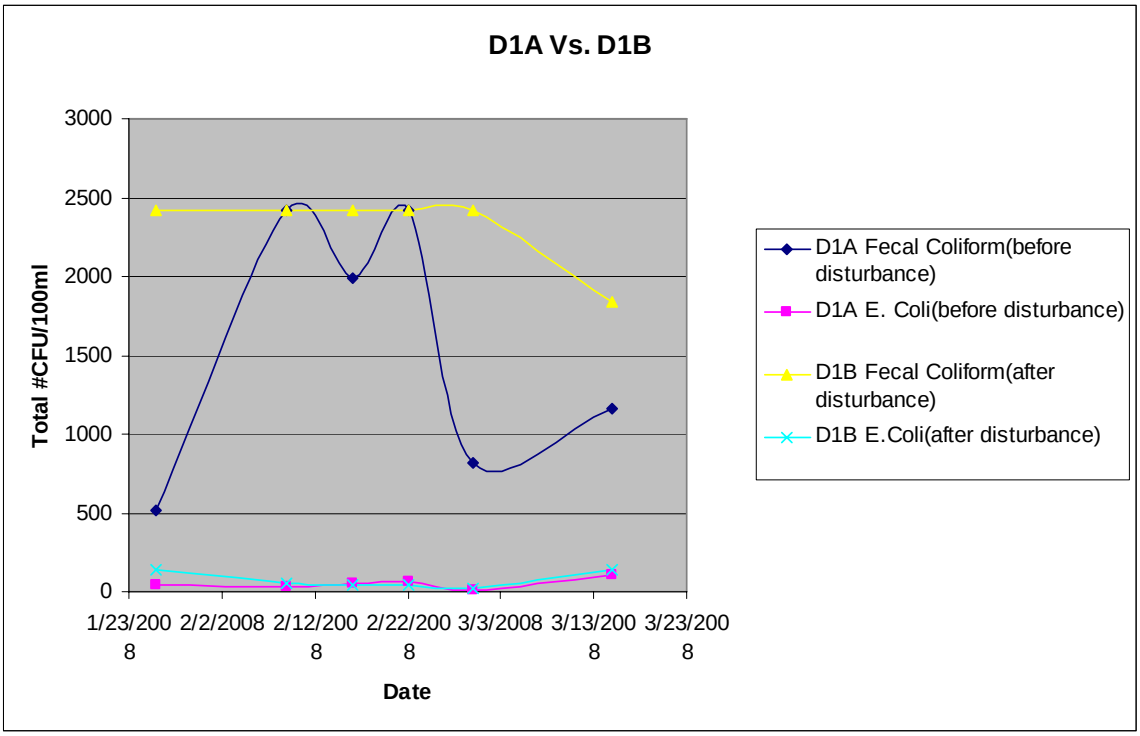
end. The first sampling site is abbreviated D1 where again the A and B mean the same as they have for all previous sampling sites. The data from this sampling site shows that after disturbance the concentrations of fecal coliform and E. coli both increased in general. This site has different qualities than the farm stream or Ellejoy stream. The farm ditch at the first sampling site had for the most part a deep pool of water behind a weir and this is where the samples were pulled. As the data shows, fecal coliform concentrations increased by a greater degree after disturbance than that of E. coli. There are many factors that could have affected the measurements at this site; the ponding of water could be more conducive for the growth and enumeration of E. coli, the lack of flowing water could have kept the E. coli in suspension not allowing them to inhabit the sediment at the bottom of the ditch, and the lack of input from another water source could have affected the concentrations of fecal coliform and E. coli. Once again the correlation between temperature and concentrations of fecal coliform and E. coli is not represented by this data set. Deposition or detachment of sediment and thus E. coli and fecal coliform could not be identified mainly due to the lack of flow at the second sampling site. On the days samples were collected, only two, at the second sampling site neither deposition nor detachment could be proven.

D1A

Date	Temp.	Total fecal Col.	Total E. Coli
1/26/2008	43.5	517.2	46.4
2/9/2008	55	2419.7	28.8
2/16/2008	62.7	1986.3	50.4
2/22/2008		2419.6	64.4
2/29/2008	46.7	821.2	12.6
3/15/2008	48.7	1158.8	112.6

D1B

Date	Temp.	Total fecal Col.	Total E. Coli
1/26/2008	43.5	2419.6	143.9
2/9/2008	55	2419.7	48.8
2/16/2008	62.7	2419.6	47.1
2/22/2008		2419.6	44.1
2/29/2008	46.7	2419.6	23.8
3/15/2008	48.7	1841.6	139.4



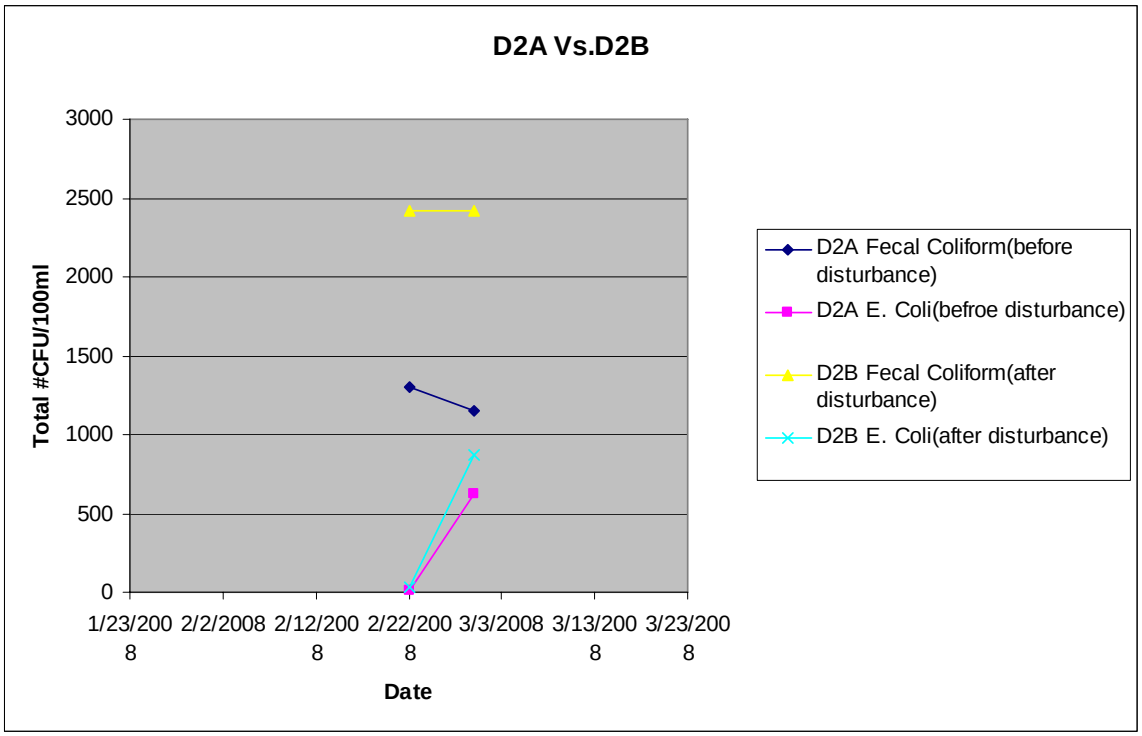
The second site that was sampled on farm ditch is abbreviated D2 and as before the A and B stand for the same thing as in all previous sampling sites. As briefly mentioned above the farm ditch did not always have water flowing past this second sampling site, so only on days that there was water flowing past this point could samples be taken. This only occurred twice and the results were of the same capacity as previous sampling sites. Here however the fecal coliform concentrations increased by a greater magnitude after disturbance than E. coli. This makes sense because fecal coliform concentrations increased more at the first sampling site on the farm ditch so it should be the same further downstream from this sampling site. Both fecal coliform and E. coli concentrations did increase after disturbance supporting the trend that has been shown by all other data sets. The correlation between temperature and concentrations of fecal coliform and E. coli could not be obtained at or represented by this sampling site mainly due to the lack of samples taken because of the lack of flow all the way through the ditch.

D2A

Date	Temp.	Total fecal Col.	Total E. Coli
1/26/2008			
2/9/2008			
2/16/2008			
2/22/2008		1299.7	8.6
2/29/2008	46.5	1149.6	626
3/15/2008			

D2B

Date	Temp.	Total fecal Col.	Total E. Coli
1/26/2008			
2/9/2008			
2/16/2008			
2/22/2008		2419.6	31.5
2/29/2008	46.5	2419.6	870.4
3/15/2008			



Discussion: As the data showed in the results section, all of the null hypotheses were disproved. The data showed with absolute clarity that the streambed sediment does have residual fecal coliform and E. coli in it. This can be said with certainty because besides a few outliers in the data, every time the streambed sediment was disturbed the concentrations of fecal coliform and E. coli were both increased. This hypothesis was important to disprove because of the implications of future land use at Ellejoy farm. Initially, the streambed sediment is sediment contained on the land, and as runoff from storm events runs across the surface of the farm soil particles are detached, transported, and deposited in the water bodies surrounding Ellejoy farm. The more sediment that is

eroded from the land ultimately means more streambed sediment. The data proved fecal coliform and E. coli inhabit the streambed sediment and it acts as a reservoir, so the more sediment that is on the streambed the higher the capacity of this sediment has for fecal coliform and E. coli. The second null hypothesis, fecal coliform concentrations are related to streambed sediment depth, was harder to identify within the data. However, I do believe the data collected shows some correlation between the two. The first sampling site on Ellejoy stream had significantly more streambed sediment than either of the other two sampling sites on Ellejoy stream. The second and third sampling sites had mainly bedrock on the streambed with only slight accumulations of sediment in the crevasses of the bedrock. Initial samples taken before disturbing this sediment contained higher concentrations of fecal coliform and E. coli than the first sampling site. After disturbing the streambed sediment the percent change from initial samples to samples taken after the streambed sediment had been disturbed was in general higher for the first site compared to the second and third sampling sites on Ellejoy stream. This seems to support a correlation with fecal coliform and E. coli concentrations and streambed depth. More extensive sampling for a longer period of time would be needed to prove this correlation. The data for all six sampling sites do not show any definite correlation between temperature and fecal coliform and E. coli concentrations. Again, sampling more extensively and for a longer period of time may uncover some relationship between the two.

The Tennessee Department of Environment and Conservation and the Tennessee Water Quality Control Board states that a concentration of 630 colony forming units of E. coli per 100 milliliters of water is considered the maximum level and 1000 colony

forming units of fecal coliform is considered the maximum level. Streams that contain concentrations at these levels or higher are considered impaired. Ellejoy stream during the sampling period was consistently above the maximum concentration for fecal coliform and only on a few sampling dates did the concentrations of E. coli reach the maximum concentration. After disturbing the streambed sediment the concentrations of fecal coliform were again consistently above the maximum concentration permitted by Tennessee standards: thus, Ellejoy stream is impaired in terms of fecal coliform. The concentrations of E. coli after disturbing the streambed sediment did occasionally reach the maximum concentrations permitted; four samples out of twenty-six total samples of water from water bodies surrounding Ellejoy farm reached the maximum concentrations. From this data it would seem that these water bodies are not impaired in terms of E. coli: however, if more extensive sampling over a longer period of time was done on these water bodies it may be found that they are impaired in terms of E. coli. The installation of the proposed University of Tennessee dairy farm would only exacerbate the impairment of these water bodies. Fecal coliform and E. coli are both used for indicators that water has come in contact with excrement of either animal or human origin. Adding large concentrations of dairy cow onto this farm would allow for the runoff running over this land to come into contact with cow feces more often, thus increasing the concentrations of fecal coliform and E. coli in the water bodies surrounding this farm. Since these streams are already on the 303d list for impaired water bodies it does not seem to be a good idea to install this dairy farm. This assessment of the future land use would be more accurate with more extensive sampling done for a longer period of time.

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