Presence and Growth of *Escherichia Coli* in Soil and Surface Water Runoff from Logs at Three Sawmill Sites in Mooresville, Indiana

Senior Research Project

Advisor: Bill Jones

June 18, 2008

By: Tarren Musgrove

Abstract

Fecal indicator bacteria, such as *E. coli*, are used as indicators of the presence of fecal material in the environment (Karns and Gagliardi, 2002). Although some *E. coli* strains can survive in the environment for long periods of time, it is thought that the cells can be generally stressed, but can also adapt easily in the environment with the appropriate environmental conditions (Alm et al., 2007; Fenlon et al. 2002). Several studies have shown that sediments serve as reservoirs for fecal pathogens and can live up to 19 weeks in the soil (Burton et al., 1987; Ingham and Lau, 2001). Saturated soil conditions may also enhance the growth of coliforms compared with indigenous flora, since coliforms are facultative anaerobes and oxygen is limited in a saturated soil (Karns and Gagliardi, 2002). *E. coli* particularly can double in growth if environmental conditions such as high moisture, warm temperature, and abundant nutrients persist consistently (Artz et al., 2005; Hicks et al., 2005). In this experimental study, three sawmill sites in Mooresville, Indiana were particularly studied for *E. coli* contamination in the soil and water runoff from their stored logs. While these sites had favorable conditions for *E. coli* to grow, it was apparent that the *E. coli* persisted and grew in the soil and water runoff from the logs. Differences in *E. coli* counts at the sites were determined by certain factors such as the amount of impervious surface, how many logs they received during certain times of the year, where they received their logs from whether it was straight from the forest or from another sawmill company, and favorable environmental conditions.

Introduction

Escherichia coli is a type of fecal coliform bacteria commonly found in the intestines of warm-blooded animals and humans (Alm et al., 2007; Rogers and Smith, 2007). The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination (Alm et al., 2007). Although most strains of *E. coli* are harmless, certain strains can produce a powerful toxin and can cause severe illness (Alm et al., 2007). There have been few systematic studies of the survival of *E. coli* in the environment, particularly in the terrestrial environment (Alm et al., 2007; Byappanahalli et al., 2006). The occurrence of *E. coli* is not limited to warm climates (Byappanahalli et al., 2006). Several studies have shown that sediments serve as reservoirs for fecal pathogens (Burton et al., 1987). Limited observations, however, suggest considerable survival periods in cattle feces, silage, soil and municipal water (Fenlon and Wilson, 2000; Fukushima et al., 1999; Maule, 1999). *E. coli* definitely persists in the primary habitat, which is either in humans or warm-blooded animals, but some recent studies have shown that *E. coli* can persist consistently in the external environment outside of the host (Alm et al., 2007; Byappanahalli et al., 2006; Hicks et al., 2006). For example, studies conducted by some scientists have suggested that some strains of *E. coli* can persist in temperate soils, freshwater beaches, and tropical and subtropical beaches (Alm et al., 2007; Byappanahallit et al., 2006; Hicks et al., 2006). But its distribution, relative abundance and persistence over time in this environment has not been fully explored. It is possible that pathogens that are present at low levels in water multiply when they are exposed to favorable environmental conditions (Gagliardi and Karns, 2002). These favorable conditions include high moisture, warm temperature, the amount of sunlight, few competitive organisms, abundant nutrients, and temperate or tropical soils (Artz et al., 2005; Hicks et al., 2006). These conditions can influence and determine the survival of *E. coli* in soil and water.

Most studies that have examined *E. coli* in soils were done in tropical, subtropical, or moderate temperate environments. Sawmills have certain environmental factors such as high moisture, warm temperature, abundant nutrients, and temperate soils for *E. coli* to persist and survive. Consequently, the objective of this study was to determine whether *E. coli* can persist and grow in the soil and water runoff from the logs at the sawmill companies located in Mooresville, Indiana.

Materials and Methods

Field investigations were performed at three sawmill sites located in Mooresville, Indiana. The sites were Wooley Lumber Company, Thiesing Veneer Company, and G.R. Wood Inc. The basic operation of sawmills is creating high quality lumber from their stored logs. Prior to creating the lumber, the stored logs are sprayed with water to prevent cracking and deterioration of the logs. In addition, watering the logs helps improve the quality of the lumber.

Site Description

At Wooley Lumber Company, the logs are stored on a concrete surface located west and east of the storm water catch basins (1554, 1555, 1556) and also near the back of the facility (Figure 1). Water samples were taken directly from the surface water runoff from the logs before it reached the catch basin in order to get the most accurate E. coli reading (Figure 1). In addition, the soil samples were collected on the west and east side of the site. The logs are usually stacked on top of each other in order to increase space for other logs. It can vary when they receive logs. For example, one of the employees mentioned that sometimes they receive new logs weekly, bi-weekly, or monthly depending on the time of the year. He said they receive an abundance of logs before it gets cold and they stop watering. Occasionally, they do move some logs around when they receive new logs. In addition, they receive their logs straight from the forest.

Figure 1. Wooley Lumber Company

At Thiesing Veneer Company, the logs are stored in the back of the facility on concrete, an impervious surface (Figure 2). The logs are stacked on top of each other, and they do not receive as many logs as Wooley Lumber. Unlike Wooley, they usually receive their logs from other log companies. During each sample event, the water and soil samples were taken at the same location (Figure 2). The water samples were taken from a filtered basin located at the end of two culverts (Figure 2). The water drained to this basin from the west and east culverts. In addition, wood chips are located between each log pile.

Figure 2. Thiesing Veneer Company

At G. R. Wood Incorporated, the logs are stored on bare soil, a pervious surface (Figure 3). Being a pervious surface, this may allow materials to infiltrate into the soil. Also, the site could be considered a depression in the ground where most of the water stayed consistent in the area (Figure 3). Most of the time, the logs are stacked on top of each other in order to allow for more space for other logs. Water samples were collected where all of the combined water runoff from the logs collected in a pipe (Figure 3). In addition, they do not receive as many logs as Wooley Lumber and receive about the same as Thiesing Veneer. They do receive a combination of logs from other log companies and straight from the forest.

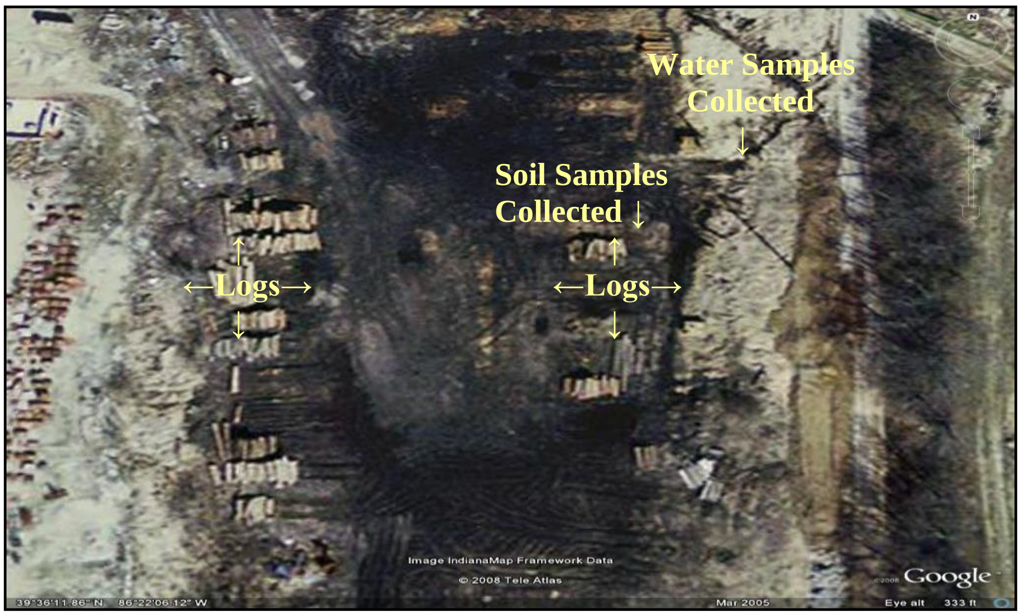

Figure 3. G. R. Wood Inc.

At Wooley Lumber, water samples were taken from August to February and soil samples were taken mostly from August to December. Wooley Lumber was the most frequently sampled site, because it had the highest *E. coli* counts. At the Thiesing Veneer and G.R. Wood sites, soil and water samples were taken from August to October or November in order to confirm if there was any *E. coli* contamination.

Water and soil samples were collected regularly either weekly or bi-weekly for the first two months to confirm that *E. coli* was present at the three sawmill sites. After the first two months, samples were collected monthly to determine if the *E. coli* would persist. The water samples were collected from the surface runoff from the logs, and soil samples were collected directly from the logs in the same area. When the soil was collected each time, it is likely that the logs were different. This might be due to the fact that logs came and gone. This is important to consider, because if new logs were sampled each time, then this would show an increase in coliform concentrations, depending on how they were cut and dragged. Each sample was collected and placed aseptically in sterile jars or plastic bags, stored at 4 degrees Celsius in an insulated container and transferred to the laboratory within two hours. Samples were tested on the day of arrival and analyzed the next day.

There are many methods that can be used to test *E. coli* in water. One specific method used was the Colilert method with the IDEXX Quanti-Tray/2000. To analyze for *E. coli* with this kit, a packet of reagent is added to 100 mL of sample water or a diluted sample and mixed by shaking. The mixed sample is then poured into a Quanti-Tray and sealed. The Quanti-Tray is then incubated at 35 degrees Celsius and read after 24 hours. A UV light is used to determine the number of fecal coliform and *E. coli* colonies based on the number of wells in the tray that show yellow/fluorescence. A conversion table comes with the kit to determine the most probable number (MPN) of *E. coli* colony-forming units per 100 mL of water (IDEXX Laboratories).

Results

E. coli was tested and found present at the three sawmill sites. Wooley Lumber was the particular site of interest, because it had the highest *E. coli* count in the water and soil. The *E. coli* colony-forming units (CFU) per 100 milliliters increased rapidly from August to October, and then decreased from October to February. These differences may, in part, be related to nutrient availability, temperature, and the amount of moisture present. From catch basin 1554, the *E. coli* count was 986 CFU per 100 mL in August and increased to 1733 CFU per 100 mL in October (Figure 4). It then decreased to 7 CFU per 100 mL in February (Figure 4).

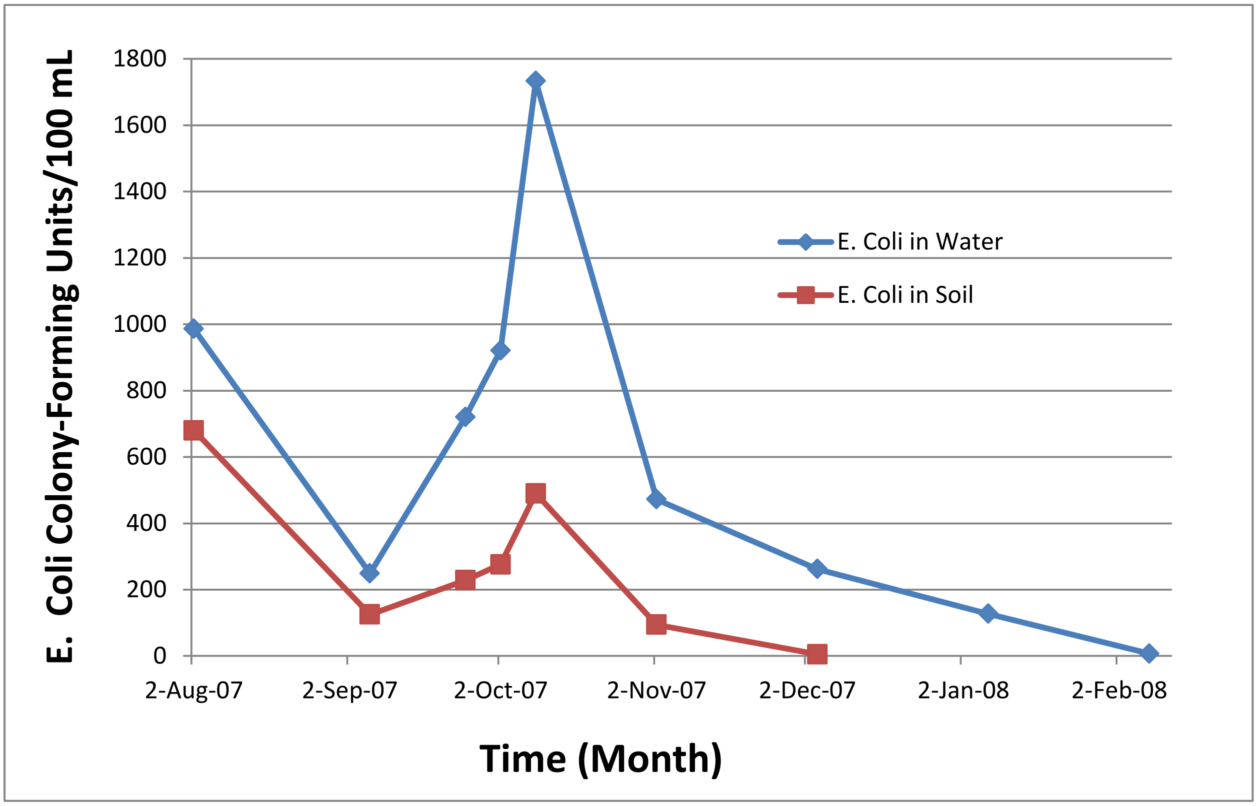

Figure 4. *E. coli* counts from catch basin 1554 at Wooley Lumber Company.

But from catch basin 1555, the *E. coli* count was 770 CFU per 100 mL in August and it increased to 2420 CFU per 100 mL in October (Figure 5). Subsequently, the count decreased to 8 CFU per 100 mL in February (Figure 5). The highest *E. coli* count in the water was approximately 2420 CFU per 100 mL from catch basin 1555 (Figure 5) and the lowest count being approximately 7 CFU per 100 mL from catch basin 1554 (Figure 4). The soil *E. coli*

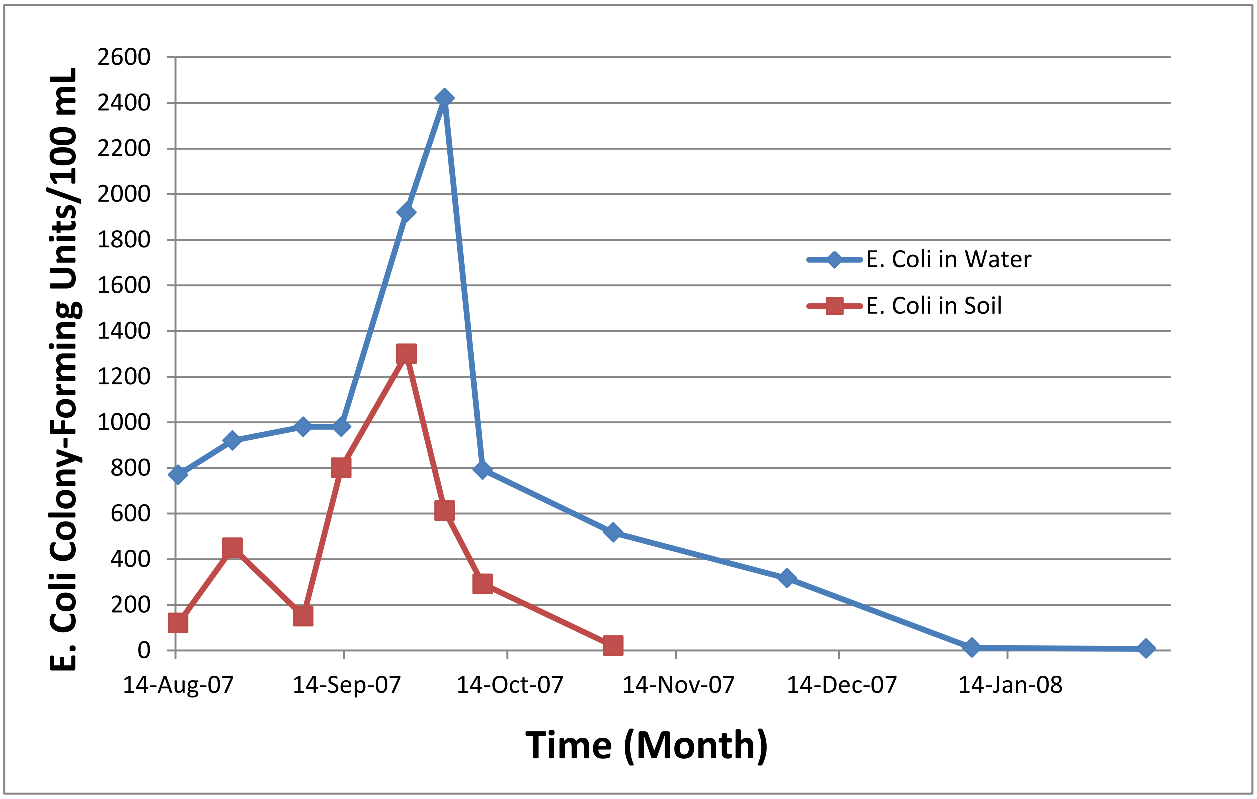

Figure 5. *E. coli* counts from catch basin 1555 at Wooley Lumber Company.

counts were not as high as the *E. coli* counts in water, but it still confirmed that the *E. coli* survived and persisted in the soil on the logs. The soil samples at Wooley Lumber were collected from the west and east side of the site. The soil samples that were collected from the west were correlated with catch basin 1554 and 1556, because they were located near the west logs. The soil samples that were collected from the east were correlated with catch basin 1555, because it was located near the east logs. For example, the soil *E. coli* count in August was 680 CFU per 100 mL, which was less than the water count at 986 CFU per 100 mL from catch basin 1554 (Figure 4). The soil counts ranged slightly, and confirmed *E. coli* was present in the soil. From catch basin 1556, samples were collected in August, September, October, and January in order to confirm if *E. coli* persisted in the water runoff (Figure 6). The *E. coli* count in August was 1300 CFU per 100 mL, and then in January it decreased to 20 CFU per 100 mL (Figure 6).

Again, the soil counts were not as high as the water counts, but this confirmed that most of the *E. coli* persisted in the soil. For example, the soil count in August was approximately 540 CFU

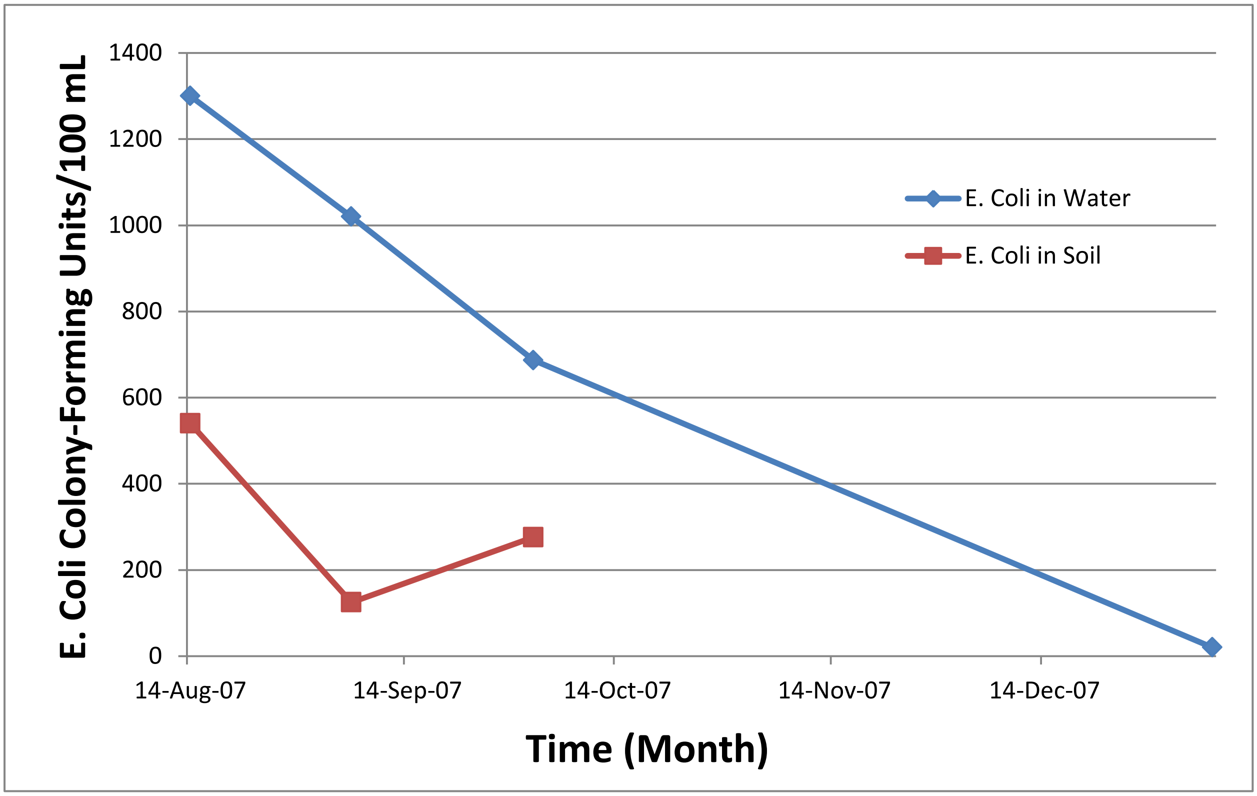

Figure 6. *E. coli* counts from catch basin #1556 at Wooley Lumber Company.

per 100 mL, which was not as high as the water count at 1300 CFU per 100 mL. Figures 4 - 6 conclude that the water and soil lines similarly correlate with each other. As a result, this demonstrates that the soil is the source of the *E. coli*.

At Thiesing Veneer, the *E. coli* water counts were not as high as Wooley Lumber. Samples were collected from September to November in order to confirm if *E. coli* also persisted at this sawmill site. The results confirmed that *E. coli* both persisted in the water and soil, but in smaller concentrations. On August 10, 2007, the water *E. coli* count was 914 CFU per 100 mL,

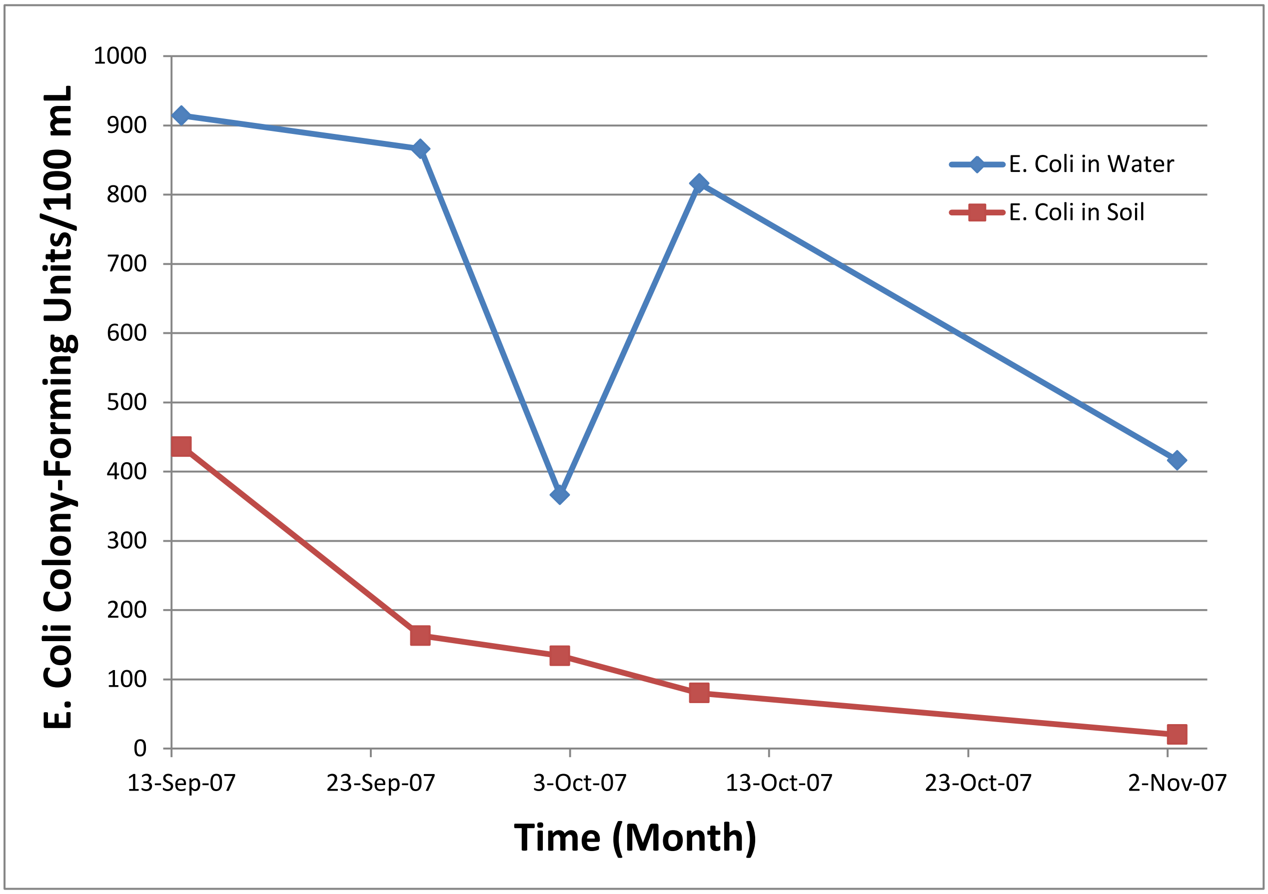

Figure 7. *E. coli* soil and water counts from Thiesing Veneer Company.

but then it decreased slightly to 366 CFU per 100 mL on October 2, 2007 (Figure 7). On October 16, 2007, *E. coli* concentrations slightly increased to 816 CFU per 100 mL, and finally the count decreased to 416 CFU per 100 mL in November (Figure 7). There could be many reasons why the count decreased on October 2, 2007. For example, it could have been a bad sample. Again, the soil *E. coli* counts were not as high as the water *E. coli* counts. The soil *E. coli* count in August was approximately 436 CFU per 100 mL, and in November it was 20 CFU per 100 mL (Figure 7). Therefore, the soil and water counts confirmed that *E. coli* persisted in the soil and water runoff. These differences may, in part, be related to nutrient availability, temperature, moisture, the amount of impervious surface, and the amount of soil on the logs. It can be assumed that more soil containing *E. coli* on the logs would result in higher

E. coli counts, but other factors such as pervious or impervious surface should be taken into account.

At G.R. Wood Incorporated, the *E. coli* water and soil counts were definitely not as high compared to the other two sites. Samples were collected from August to November. *E. coli* was found in both the soil and water. In August, the *E. coli* water count was approximately 378 CFU per 100 mL and interestingly, this time the soil count was higher than the water count being 727 CFU per 100 mL (Figure 8). In November, the *E. coli* water count decreased to 8 CFU

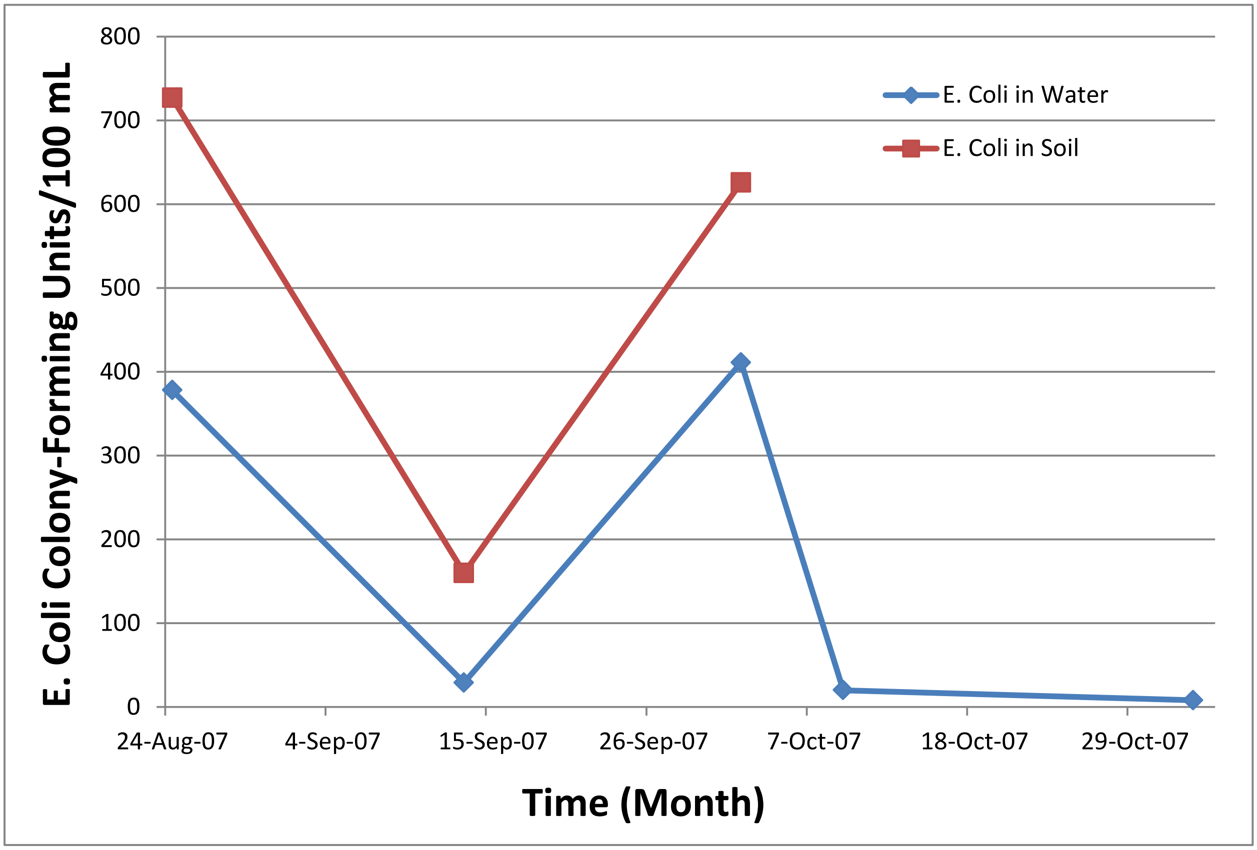

Figure 8. *E. coli* soil and water counts from G.R. Wood Incorporated.

per 100 mL (Figure 8). However, throughout the sampling months, the *E. coli* count in the soil and water fluctuated. This may, in fact, be due to the differences in temperature, moisture, nutrient availability, and the amount of soil on the logs. In fact, G.R. Wood's soil count was always higher than the water count, and this may be due to the amount of pervious surface located at this sawmill site. Pervious surface can affect the count of the *E. coli*. The *E. coli* can percolate into the soil, which can affect the concentration of the *E. coli* in the water. As a result, the water count would be lower in concentration than the soil count.

Discussion

Investigation of E. coli

In the current study, some investigation was done to confirm if *E. coli* was coming from the soil on the logs and/or from other sources such as sewer leaks or animal feces. First, a dye test was done in the toilet located at Wooley Lumber's building. The dye test was confirmed negative, because the dye was seen in the sanitary sewer line. As a result, the *E. coli* problem could not have been coming from a sanitary sewer leak. After the dye test, it seemed feasible to test the soil on the logs. The test confirmed that *E. coli* was present in the soil (Figures 4 - 8). Studies have shown that certain strains of *E. coli* can persist and grow in temperate soils (Alm et al., 2007; Byappanahalli et al., 2006; Hicks et al., 2006). There could be many reasons why the soil may be the source of the *E. coli*. However, it only seemed logical to presume that the logs were dragged through the surface temperate soils in Indiana that already contained *E. coli* for some time. Recent evidence suggests that the *E. coli* in these temperate soils most likely came from different sources whether human or non-human and as a result, the *E. coli* adapted to the environment (Alm et al., 2007; Byappanahalli et al., 2006; Hicks et al., 2006). At the same time, other sources of *E. coli* should be considered in this process such as animal feces from geese, ducks, squirrels, and other animals that could possibly contribute to the *E. coli* contamination. I asked the owner at the Wooley Lumber site if he noticed many animals roaming around. He mentioned that there were not too many animals that he noticed. He also commented that every so often they would find dead animals between the logs, but not enough to contribute to the problem.

Persistence and Survival of E. coli

Escherichia coli has been routinely used as an indicator of fecal contamination in drinking and recreational waters throughout the world (Byappanahalli et al., 2006). It is mainly a member of the gastrointestinal tract of warm-blooded animals and humans, which is considered the primary habitat (Alm et al., 2007; Byappanahalli et al., 2006; Hicks et al., 2006). It is often considered to be a temporary member of the microbiota found in water and soil, although recent evidence suggests that certain *E. coli* strains can persist in temperate soils, freshwater beaches, and tropical and subtropical soils, which is considered the secondary habitat (Alm et al., 2007; Byappanahalli et al., 2006; Ishii et al., 2006). Once *E. coli* comes in contact with soil either from human or warm-blooded animal fecal inputs, the *E. coli* can become naturalized. It can then adapt to the environment. Ishii and colleagues (2006) have proposed the term 'naturalized' strains to refer to the persistent *E. coli* genotypes that comprise the autochthonous members of the microbial community in the environment, and these naturalized *E. coli* strains are a significant component of the environmental coliform microbiota. Data from studies have suggested that soilborne, naturalized *E. coli* strains can adapt to the soil when *E. coli* was not continuously added from external sources (Hicks et al., 2006). Since heat was consistently provided in the upper surface of the soils, this promoted *E. coli* growth (Hicks et al., 2006). As a result, it can maintain a stable population density after growth in soil (Hicks et al., 2006). However, certain factors in the secondary habitat such as UV radiation, temperature, and predation have shown to decrease the concentration of specific individual strains (Alm et al., 2007). But many studies have shown that *E. coli* can persist for several weeks in soil (Ingham and Lau, 2001). It can persist and survive from 105 days to 19 weeks depending on certain environmental factors such as the amount of sunlight, nutrients, moisture, predation, and other factors (Ingham and Lau, 2001; Fenlon et al., 2002). It has been shown that *E. coli* can persist 19 weeks if the soil temperature ranges from 9 - 21 degrees Celsius (Ingham and Lau, 2001). Also, temperate soils can significantly affect indicator bacteria survival, and studies have shown that certain *E. coli* strains can persist and grow in temperate soils (Byappanahalli et al., 2006; Hicks et el., 2006). Figures 4 - 8 show that *E. coli* has persisted in the soil and most likely came from the temperate soils based on evidence from environmental studies. As a result, the *E. coli* in the soil from the logs could continue to grow if the favorable environmental conditions persist.

Favorable Environmental Conditions

At the three sawmill sites, the *E. coli* appeared to persist and/or grow if there were favorable conditions (Byappanahalli et al., 2006; Hicks et al., 2006). There were many favorable conditions at these sites such as good moisture, available nutrients, warm temperatures, and a good habitat to grow. Therefore, this would allow for the *E. coli* to grow consistently (Ishii et al., 2006). From Figures 4 - 8, *E. coli* consistently persisted from July to October in high counts. In this time period, the water was constantly being sprayed on the logs, which persistently added moisture to the site. Although soil moisture was not identified as a main explanatory variable of background *E. coli* numbers in soil in this field investigation, its potential importance as a factor influencing the survival of *E. coli* in the environment cannot be discounted (Artz et al., 2005). For example, one study suggested that soil ecological processes are important in regulating populations of enteric indicator bacteria, such as *E. coli* (Rogers and Smith, 2007). In general, logs usually contain an abundant of organic matter from the bark and other materials on the logs. Studies have shown that *E. coli* would grow the fastest if the soil had a relatively large amount of organic matter, and more slowly in soils having less organic matter (Hicks et al., 2006). In addition, having warm to hot temperatures at these sites helped increase the conditions for *E. coli* to grow and persist in the soil. At the same time, the water runoff from the logs caused some of the *E. coli* to leach into the storm water system.

After October, the temperatures started to decrease. Temperature can affect the growth of *E. coli* (Hicks et al., 2006). However, some studies have shown that certain strains of *E. coli* has the capability to not only survive in low concentrations for long periods at low temperatures (3 months at 4 degrees Celsius), but also grows at 6 degrees Celsius (Jones, 1999). From November to February, temperature and moisture most likely affected the growth of *E. coli* at these sawmill sites (Figures 4 - 8). For example, from catch basin 1554, in October the *E. coli* count was 1733 CFU per 100 mL and in December, it was 262 CFU per 100 mL (Figure 4). This count greatly decreased from October to December by 1471 CFU per 100 mL. This is due likely because the temperature decreased from 78 degrees Fahrenheit in October to 35 degrees in December. This is a 43 degrees temperature difference, and as a result, the *E. coli* cannot grow as rapidly.

Impervious and Pervious Surface

Even though *E. coli* persisted at the three sawmill sites, not all sites had the same *E. coli* counts in the water and soil. This could be, in part, due to the fact one site might have more impervious surface than another site. Impervious surfaces can contribute to changes in quality and quantity of the water. Wooley Lumber had the most impervious surface than the other two sites. As a result, this might explain why this site had the highest *E. coli* water count (Figure 4 - 6). On the other hand, Thiesing Veneer had some impervious surface but not as much as Wooley's site. Also, the water runoff from the logs drained into a culvert located both on the east and west side, and finally into one large basin. As a result, this could have affected the *E. coli* concentration. At the same time, it could have decreased in concentration by the wood chips acting as a buffer between each log stack and grass nearby. Therefore, this could be one explanation of why the *E. coli* counts were not as high as Wooley's site. However, G.R. Wood Inc. has a pervious surface, which greatly affected the *E. coli* water counts (Figure 8). A pervious surface can allow materials such as the *E. coli* to percolate into the soil layer. At this site, the logs practically sat in a fairly deep depression in the ground allowing the *E. coli* to either stay relatively consistent in the area or percolate into the soil. As a result, the *E. coli* water counts were fairly low. In fact, the *E. coli* soil counts were quite higher than the water counts (Figure 8). This may be because the pervious surface affected the concentration of the *E. coli* in the water. One theory that should be considered is that the concentration decreased from the soil on the logs to the water on the pervious surface due to the fact that the *E. coli* percolated into the soil rather than flowing directly into the pipe where the water samples were collected.

Shipment of Logs

While considering impervious and pervious surface runoff as a factor affecting the *E. coli* counts, other factors should also be considered in this study. Another factor that could affect the difference in *E. coli* counts at the sites is the difference in concentration levels of logs during certain times of the year. Prior to being a professor at Indiana University, Dr. Burney Fisher worked for a forestry company for quite awhile and later served as the State Forester. He mentioned that during certain times of the year that sawmill companies receive more shipments of logs usually before winter. This is because they usually want to earn more productivity before it gets cold. From Figures 4 - 8, each sawmill company showed a peak around October. This is due to the fact that more logs are shipped around this time of the year. Usually, more logs can indicate that more soil containing *E. coli* is present. As a result, this causes the *E. coli* count to be higher around this time of the year. He also mentioned that Thiesing Veneer's logs do not come straight from the forest, and that they usually come from other log companies where the logs have already been sprayed with water. As a result, there would be less soil on the logs, which could explain why they had lower *E. coli* counts (Figure 7). On the other hand, Wooley Lumber receives their logs straight from the forest, which means there would likely be more soil on their logs. If there is more soil present, then this could conclude why there are higher concentrated levels of *E. coli* at this site (Figure 4 - 6). This could explain why Wooley Lumber has higher *E. coli* counts than the other two sawmills.

Conclusion

Soil is a finely balanced, complex ecosystem, with high microbial diversity and significant populations of a variety of microorganisms (Rogers and Smith, 2007). These populations are involved in food webs involving viruses, bacteria, fungi, protozoa and other eukaryotic groups. While pathogenic bacteria such as *E. coli* can be added to soils through either human and/or warm-blooded animal input, the fact is that the bacteria can adapt, grow, and survive for long periods of time in soils (Alm et al., 2007; Byappanahalli et al., 2006; Hicks et al., 2006). The limited data (Figures 4 - 8) available does conclude that *E. coli* can remain viable in soil and water for considerable lengths of time. It is likely therefore that during survival in such environments, cells will encounter conditions of stress, such as nutrient starvation. This may alter the ability of *E. coli* to compete with indigenous microbial populations for nutrients during subsequent enrichment stages of detection, particularly when cell concentrations are low (Byappanahalli et al., 2006). Both abiotic (temperature, pH, soil moisture, soil type) and biotic (composition and diversity of the microbial community) factors affect the survival capabilities of bacteria introduced into the soil habitat (Artz et al., 2005; Hicks et al., 2006). Because the extent to which these factors affect survival most likely depends on interactions between the various environmental factors, the overall set of abiotic and biotic soil characteristics should be taken into account. Moreover, the existence of the *E. coli* in the water and soil at the three sawmill sites (Figures 4 - 8) confounds the use of this bacterium as a reliable indicator organism for pathogen spread and survival (Hicks et al., 2006). *E. coli* does exist at the sawmill sites and the concentration levels depend on certain factors such as the amount of impervious surface, how many logs they received during different times of the year, where they received their logs from, and favorable environmental conditions. It is conclusive that the temperate soils in Indiana are the source of the *E. coli* at these sites. Based on systematic studies, it is evident that the *E. coli* was first introduced either by human or non-human inputs and then adapted in the environment.

Mitigation

Since bacterial contamination has been detected, there could be many ways to prevent *E. coli* contamination at sawmill sites. One way would be to build a retention pond so that the water is recycled throughout the pond. Not only is this idea consuming less water, but also it is preventing contamination of *E. coli* to enter waters of the state. Another way would be to regularly rotate the logs around the site. This could greatly reduce *E. coli* concentrations levels. By moving newer logs away from the runoff area, then this could affect the *E. coli* water counts. Also, another way to prevent contamination would be to install a product called the Smart Sponge created by AbTech Industries. It is designed to remove both oil and bacteria. It can be installed in catch basin inserts. It could be costly, but it would take care of the contamination problem. However, if mitigation is considered, it should be technically feasible, cost-effective and environmentally sound.

References

Alm, E.W., L.M. Calhoun, J.M. Mlandonicky, S.T. Walk, and T.S. Whittam. 2007. Genetic diversity and population structure of *Escherichia coli* isolated from freshwater beaches. Environmental Microbiology. 9:2274-2288.

Artz, R.E., K. Brown, K. Killham, W. Towers, and J. Townend. 2005. Soil macropores and compaction control the leaching potential of *Escherichia coli* 0157:H7. Environmental Microbiology. 7:241-248.

Burton, G.A., Jr., D. Gunnison, and G.R. Lanza. 1987. Survival of pathogenic bacteria in various freshwater sediments. Applied Environmental Microbiology. 53:633-638.

Byappanahalli, M.N., S. Ishii, D.A. Shively, M.J. Sadowsky, and R.L. Whitman. 2006. Population structure, persistence, and seasonality of autochthonous *Escherichia coli* in temperate, coastal forest soil from the Great Lakes watershed. Environmental Microbiology. 8:504-513.

Fenlon, D.R., N.F. Hepburn, M. Macrae, T.H. Pennington, I.D. Ogden, S.M. Rusbridge, and N.J.C. Strachan. 2002. Long-term survival of *Escherichia coli* 0157 on pasture following an outbreak associated with sheep at a scout camp. Applied Microbiology. 34:100-104.

Fenlon, D.R., I.D. Ogden, I. Svoboda, and A. Vinten. 2000. The fate of *Escherichia coli* 0157 in cattle slurry after application to land. Journal of Applied Microbiology. 88S:149S-156S.

Fukushima, H., K. Hoshina, and M. Gomyoda. 1999. Long-term survival of Shiga toxin-producing *Escherichia coli* 026, 0111, and 0157 in bovine feces. Applied and Environmental Microbiology. 65:5177-5181.

Gagliardi, J.V., and J.S. Karns. 2002. Persistence of *Escherichia coli* 0157:H7 in soil and plant roots. Environmental Microbiology. 4:89-96.

Hicks, R.E., S. Ishii, W.B. Ksoll, and M.J. Sadowsky. 2006. Presence and growth of naturalized *Escherichia coli* in temperate soils from lake superior watersheds. Applied and Environmental Microbiology. 72:612-612.

IDEXX Laboratories, Inc. 2008. Maine, NE, USA. Accessed online: <http://www.idexx.com/ water/colilert/>

Ingham, S.C., and M.M. Lau. 2001. Survival of faecal indicator bacteria in bovine manure incorporated into soil. Applied Microbiology. 33:131-136.

Jones, D.L. 1999. Potential health risks associated with the persistence of *Escherichia coli* 0157 in agricultural environments. Soil Use and Management. 15:76-83.

Maule, A. 1999. Environmental aspects of *E. Coli* 0157. International Food Hygiene. 9:21-23.

Rogers, M. and S.R. Smith. 2007. Ecological impact of application of wastewater biosolids to agricultural soil. Water and Environment Journal. 21:34-40.