Relationships Between Land Uses and Indicator Bacteria in a Riverine Environment

Louwanda W. Jolley[1], Jeremy W. Pike[2], William R. English[3] and John C. Hayes[4]

AUTHORS: [1]Research Specialist III, Department of Forestry and Natural Resources, Clemson University; [2] Research Specialist III, Department of Forestry and Natural Resources, Clemson University; [3] Associate Professor, Department of Forestry and Natural Resources, Clemson University; [4] Professor, Department of Agriculture and Biological Engineering, Clemson University

REFERENCE: Proceedings of 2008 South Carolina Water Resources Conference, October 14-15, 2008 at the Charleston Area Convention Center, ISBN: 978-0-615-25592-7

Abstract. The two most commonly cited causes of water quality degradation in the United States are fecal coliform bacteria and sediments. Increased levels of indicator bacteria in streams have been linked to land uses. Stream bottom sediments have been shown to act as reservoirs for indicator bacteria and pathogenic bacteria. Two streams in South Carolina were studied to examine the relationship between the levels of indicator bacteria in resuspendable bottom sediments and the overlying water and the effects of land use on resuspendable bottom sediment levels of indicator bacteria. The indicator bacteria used for this study was fecal coliform. At base flow, fecal coliform levels in the resuspendable bottom sediments were hundreds to thousands of times greater than levels in the overlying water, $p<0.0001$. Water and resuspendable bottom sediment fecal coliform levels were related, $r^2=0.5342$. At base flow water levels of fecal coliforms were not related to land use s, $p=0.1163$, but resuspendable sediment levels were, $p= 0.0283$. Bacteria associated with bottom sediments are resuspended and transported with the sediments when the sediments are disturbed by rain events and a variety of water related activities. Therefore, relationships between fecal coliform levels, total suspended solids (TSS), and land uses were investigated during rain events. Fecal coliform levels in water were related to both TSS values, $p<0.0001$, and land uses, $p=0.0164$.

BACKGROUND AND RELATED WORK

Bacterial contamination adversely affects water quality as a result of its potential for causing serious human health problems. Although fecal coliform is no longer the indicator bacteria recommended by the United States Environmental Protection Agency (USEPA,1986), fecal coliform is the indicator bacteria used by South Carolina and a number of other states, and therefore was used for this study. Indicator bacteria are used to determine the possible presence of organisms that are pathogenic to humans. Currently only surface water counts of these bacteria are commonly measured by federal and state water quality agencies. The association of indicator bacteria with resuspendable bottom sediments is not considered.

Free-living bacteria in the water column are extremely small and have very slow settling rates (Gannon et al., 1983). When bacteria are adsorbed to sediment particles, they settle to the bottom at a faster rate (Gannon et al., 1983; An et al. 2002). These findings are supported by others who show counts of fecal coliform bacteria in sediments exceeding those in overlying waters by 1-4 $\log_{10}$ (Burton et al., 1987; Buckley et al., 1998). In addition, sediments have been shown to act as reservoirs of indicator bacteria and a number of waterborne pathogens (Howell et al., 1996; Carbill et al., 1999; LeJeune et al., 2001), and therefore, may serve as continual sources of waterborne human pathogens if they are present.

Indicator bacteria in sediment, including fecal coliforms and *E. coli*, have been demonstrated to live significantly longer than bacteria in the overlying water (Howell, 1996; LeJeune et al., 2001). Davies et al. (1995) showed that sediments provide a more nutrient rich environment to support growth of bacteria. Longer survival rates and growth have been attributed to several factors including sediment size and composition, temperature, nutrients and protection from predators (Sherer et al., 1992; Howell et al., 1996). Resuspension of fecal coliform bacteria from sediment reservoirs results in water quality degradation in the affected areas (An et al., 2002; Jolley, 2005) and can occur as a result of storm flows, swimming, boating and other activities. Resuspended sediments become convenient vehicles for the transport of bacteria (Brettar and Hoffe, 1992; Kistemann et al., 2002) into lakes, reservoirs and bays.

Numerous studies of the relationship between land use and water quality have found that bacterial quality of water is directly affected by land use (Tong et al., 2002; Jamieson et al., 2004). Jamieson et al. (2004) stated that there are two principle stores of bacteria; the land store and the stream store. Rainfall and runoff mobilize the land store and resuspension mobilizes the stream store.

The goals of this research were to quantify the relationship between resuspendable bottom sediments and surface water levels of fecal coliform during base flow and during resuspension of bottom sediments by rain events. Land uses have been shown to affect surface water counts of fecal coliform bacteria at base flow (Tong et al., 2002); therefore the influences of land use on resuspendable bottom sediment levels of fecal coliform bacteria were investigated at base flow. During rain events, levels of fecal coliforms in water have been shown to be significantly higher than at base flow (Jolley, 2005). Resuspension of bottom sediments and fecal coliforms also occurs (McDonald et al., 1982) and soils with associated bacteria are carried to streams by runoff. Therefore, the relationships of fecal coliforms, total suspended solids (TSS), and land uses were studied during rain events.

EXPERIMENTAL DESIGN AND METHODS

The sampling sites were located on the Saluda and Reedy Rivers and selected tributaries in South Carolina, USA. Both rivers are located in the upper half of the Saluda River Basin. The 30 year rain fall average for the area is about 13 cm and the average daily temperature is 15.4°C. Soils are moderately deep, well drained, with clayey red to yellowish brown subsoils and erodibility K factors of 0.22 to 0.26. Slopes for this half of the basin range from 2%- 80% with the average slopes for individual sub watersheds ranging from 15% to 25% (SCDHEC, 2004). Land use was determined with aerial photos and ground proofing and was classified based on definitions established by the U. S. Geological Survey's National Land Cover Data. Three categories were selected based upon per cents forest, agriculture, and urban land use for the sub watersheds (SCDHEC, 2004). Twelve sites were chosen and were categorized as follows:

1. Forested/agriculture land use was 86% or greater combined forest and agriculture land uses and 10% or less urban land use.
2. Urbanizing land use was 13% - 20% urban land use.
3. Urban land use was 30% or greater urban land use.

Paired water and bottom sediment samples were taken monthly during base flow, at each site, for one year to compare fecal coliform counts in surface water and resuspendable bottom sediments. Base flow was defined as having no precipitation for five days before sampling. Water was sampled by taking a grab sample approximately 1 m from shore and approximately15 cm below the surface. The sediment sampling sites were approximately 1 m² with the water sampling site as the center. Samples were taken by aspirating the top 1-2 cm of the bottom sediments. The first sediment sample was taken from the bottom sediments directly below the water sampling site. The other three sampling sites were randomly located within the 1m^2. Four samples were taken within the 1 m² square and combined in a sterile container.

Rainfall events were studied as a natural source of resuspension and transport of bottom sediments. Seven rainfall events were sampled at three of the land use sites. Rainfall for the events ranged from 2.8 - 6 cm. Each of the three land use categories was sampled at least twice. An Isco Sampler Model 3700 (Teledyne ISCO, Lincoln, NE) was used to sample each event from base flow before the rain event until the hydrograph approached base flow after the rain event. Samples were taken hourly until 6 hours after the peak of the hydrograph. The sampling interval was then expanded to 2 hours for the remainder of the hydrograph. Flow data was obtained from either USGS Gage Stations or an ISCO Flow Meter (Teledyne ISCO, Lincoln, NE).

All samples were analyzed for fecal coliforms using the membrane filter method as described in Method 9222D, Standard Methods (2000). TSS were used to measure resuspendable sediments during the rainfall events and were determined using Method 2540D, Standard Methods (2000). Resuspendable bacteria from bottom sediment samples taken at base flow were analyzed by shaking vigorously by hand (Craig et al., 2002) for one minute and sampling the supernatant immediately for fecal coliforms. Fecal coliform analysis was performed as for surface water samples. Sediment volumes were measured by allowing the sediments to settle for 24 hours in a graduated cylinder. Sediment volume included the sediment and its interstitial water. Resuspendable levels of fecal coliforms in the sediment were calculated as Colony forming units (CFUs)/100 ml of sediment for ease of comparison with surface water values. CFUs/100 ml of sediments were calculated as follows:

$$(C/D \times TV) - (CW/100 \times WV) = A$$

$$A/SV \times 100 = \text{CFUs}/\ 100\text{ml sediment}$$

Where:
C = Colony forming units on plate
CFUs = Colony forming units
D = volume of sample diluted
CW = CFUs surface water
TV = Total volume of water and sediment
WV = volume of water
SV = volume of sediment
A = Total CFUs in sediment

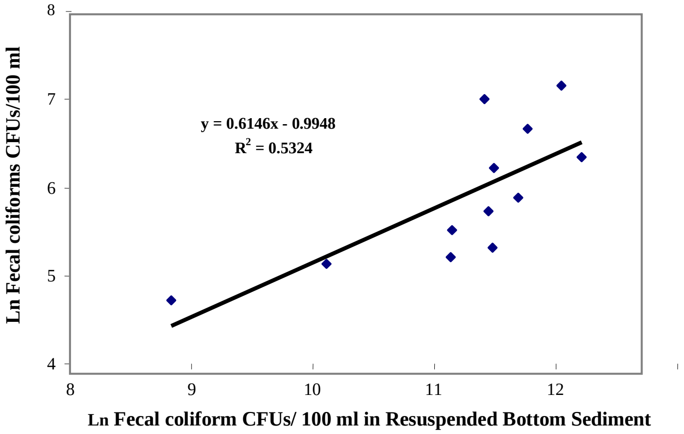
Data was analyzed using Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA) and SAS V9.1 (SAS Institute Inc., Cary, N. C.). Fecal coliform levels and TSS values were log transformed before statistical analysis.

RESULTS AND DISCUSSION

Base flow

At base flow, each of the 12 sites had resuspendable bottom sediment mean fecal coliform levels significantly higher ($p<0.001$, paired t-test) than water mean fecal coliform levels. Mean fecal coliform levels of resuspendable bottom sediments were 10 to10,000 times higher than mean fecal coliform levels for water. The relationship between log transformed surface water and resuspendable bottom sediment fecal coliform levels can best be described as $y=0.6146x - 0.9948$ with $R^2= 0.5324$ and $p<0.0001$ (Figure 1.). The R^2 for this relationship suggests that although surface water and resuspendable bottom sediment fecal coliform levels are related there are other factors involved. Some of the possible factors are land use, multiplication of bacteria in the sediments and predation of bacteria.

Figure 1. Comparison of log converted mean CFUs in surface water and log converted mean CFUs in bottom sediments at base flow.



One of the factors that could possibly affect the relationship, land use, was also investigated in this study. Three land uses, forest/agriculture, urbanizing, and urban, were used to compare surface water fecal coliform levels and resuspendable bottom sediment fecal coliform levels at base flow. Analysis of variance (ANOVA) was used to compare the land uses. Forested/agricultural, urbanizing and urban were used as treatments. Surface water fecal coliform levels were not related to land use, p = 0.1163, but resuspendable bottom sediment fecal coliform levels were related p = 0.0283. A possible explanation is that land clearing and construction in urbanizing areas is facilitating the mobilization of the land stores of bacteria referred to by Jamieson et al. (2004) during runoff from rain events. Sediments and associated bacteria are then deposited near their area of land origin (Gannon et al., 1983). Sediments and associated bacteria move down stream much more slowly during base flow than surface water and the bacteria it contains, therefore the surface water being sampled at base flow is from upstream.

The Least Squared Differences (LSD) for both the fecal coliform levels for water and the resuspendable bottom sediments for the three land uses indicated that they were not all different. Both the mean fecal coliform levels for water and mean fecal coliform levels for resuspendable bottom sediment for forested/agricultural land use were significantly different from fecal coliform levels for urbanizing and urban land uses. Fecal coliform levels for surface water and resuspendable bottom sediments for urbanizing land use and urban land use were not different. A possible explanation for the lack of significance between fecal coliform levels for water and for resuspendable sediments in urbanizing land use areas and urban land use areas is that they had similar amounts of impervious cover, urbanizing = 29.9% and urban = 32% (Center for Watershed Protection, 2003), and the urban area had construction taking place near one of the streams. The data from this study demonstrate that other factors influence bacterial water quality and that examination of surface water only is not sufficient to determine the bacterial quality of water. Resuspension of fecal coliforms from bottom sediments into water and land uses are important when considering bacterial loading of waters.

Storm flow

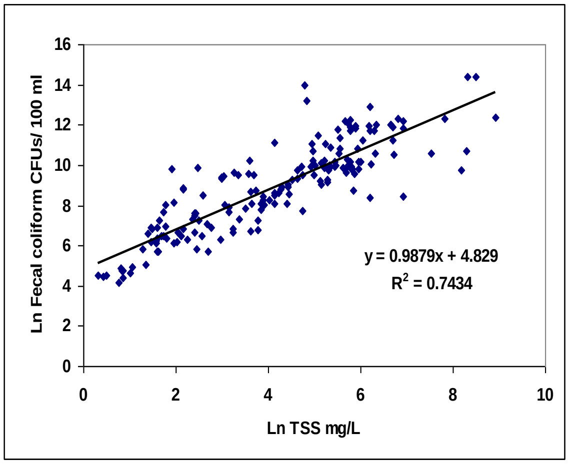
Resuspension and transport of bottom sediments and associated bacteria were studied during rain fall events. Hydrographs for the seven rain events were compared using ANOVA to determine if the results of the study were dependent upon the amount or intensity of rainfall. All the hydrographs were different, $p<0.0001$. Mean fecal coliform levels and mean TSS values were compared for each of the land use categories. Mean fecal coliform levels and mean TSS values were highly correlated for each of the land uses (Table 1). The relationship between mean fecal coliform levels and mean TSS values were evaluated for the seven rain events. A linear relationship, $r=0.8622$, $R^2=0.7434$, existed between mean fecal coliform levels and mean TSS values (Figure 2.). This suggests that fecal coliforms are adsorbed to or closely associated with the sediments and are resuspended and transported with the sediments.

The three land uses, forest/agriculture, urbanizing, and urban, were used as treatments in an ANOVA to compare water fecal coliform levels and TSS values during the seven rain events with land use and with each other. Fecal coliform levels and TSS during storm flows were significantly related to land use. Mean fecal coliform levels were related to both land uses ($p= 0.0164$) and mean TSS values ($p<0.0001$). Mean TSS values were related to land use ($p=0.0100$) and to mean fecal coliform levels ($p<0.0001$). The LSD for both the fecal coliform levels for water and the TSS values for the three land uses indicated that the three land use categories were significantly different. Mean fecal coliform levels in water and mean TSS during rain events were higher for urbanizing land use than for urban land use (Table 1.) and both were higher than forested/agriculture. Storm flow fecal coliform levels can be predicted from storm flow TSS for these watersheds (Figure 2). Urban land use had 20 times as many mean CFUs and 8 times as much mean TSS as forested/agricultural land use. Land being urbanized had 72 times as many mean fecal coliforms and 29 times as much mean TSS as forested/agricultural land and 3.5 times as many mean fecal coliforms and 3.5 times as much mean TSS as urban land use. Urban land use has traditionally been thought to be the most degrading to water quality during rain events (Paul and Meyer, 2001), but these data indicate that in the watersheds studied land that is being urbanized is contributing 72 times more fecal coliforms and 29 times as much TSS or sediment. The process of changing from less intense to more intensive land use appears to be more damaging to water quality than established land uses.

Table 1. Mean CFUs, mean TSS values and correlation coefficients for the seven rain events and three land use categories.

Land use	Mean CFUs/ 100 ml	Mean TSS mg/L	Correlation Coefficient r =
Forest/ Ag	1951	25	0.8524
Urbanizing	141532	713	0.7942
Urban	39544	201	0.8867

Figure 2. Comparison of mean TSS values and mean CFUs and regression formula describing the relationship.
n = 175 each for CFUs in water and TSS.



CONCLUSION

The data from this study demonstrates that numerous factors may be influencing bacterial water quality and that examination of surface water alone is not sufficient to determine the bacterial quality of water. Land use plays an important part in bacterial water quality and it appears that streams with urbanizing areas are the most likely to suffer from degradation. The construction that accompanies urbanization likely plays an important part in degradation of water quality because clearing of the land before construction usually disturbs the soil. The soil and accompanying bacteria a then carried into the stream by runoff. Better ways of protecting streams during urbanization need to be developed and enforced. Resuspension of fecal coliforms from bottom sediments into surface water and land use are both important factors when considering bacterial loading of waters. However, water quality degradation is seldom caused by a single factor as the data above illustrates. In the past, TSS, fecal coliforms and land use have been considered separate causes of water quality impairments, but the relationships between TSS, fecal coliforms, bottom sediments and land uses compound our lack of understanding of these impairments. The factors studied have strong statistical relationships at base flow and during storm flows and should no longer be considered separately when we consider bacterial loading in streams. These factors should be added to current bacterial loading models. Other factors, such as the multiplication of bacteria in the sediments and predation maybe important and should also be examined and quantified.

REFERENCES

An, Y. J., D. H. Kampbell, and G. P. Breidenbach. 2002. *Escherichia coli* and total coliforms in water and sediments at lake marinas. Environmental Pollution 120: 771-778.

Brettar, I., and M. G. Hofle. 1992. Influence of ecosystematic factors on survival of *Escherichia coli* after large-scale release into lake water mesocosoms. Journal of Applied and Environmental Microbiology 58:2201-2210.

Buckley, R., E. Clough, W. Warnken, and C. Wild. 1998. Coliform bacteria in stream bed sediments in a subtropical rainforest conservation reserve. Water Research 32:18852-1856.

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

Carbill, C., R. Donald, J. Snelling, R. Foust, and G. Southam. 1999. The impact of sediment fecal coliforms reservoirs on seasonal water quality in Oak Creek, Arizona. Water Research 33:2163-2171.

Center for Watershed Protection. 2003. Impacts of Impervious Cover on Aquatic Systems. Watershed Protection Research Monograph No. 1. Ellicott City, MD 21043.

Craig, D.L., H. J. Fallowfield and N. J. Cromar. 2002. Enumeration of faecal coliforms from recreational coastal sites: evaluation of techniques for the separation of bacteria from sediments. Journal of Applied and Environmental Microbiology 83: 557-565.

Davies, C. M., J. A. H. Long, M. Donald, and N. J. Ashbolt. 1995. Survival of fecal microorganisms in marine and freshwater sediments. Journal of Applied and Environmental Microbiology 61:1888-1896.

Gannon, J. J., M. K. Busse, and J. E. Schillinger. 1983. Fecal coliforms disappearance in a river impoundment. Water Research 17: 1595-1601.

Howell, J. M., M. S. Coyne, and P.L. Cornelius. 1996. Effects of sediment size and temperature on fecal bacteria mortality rates and the fecal coliforms/fecal streptococci ratio. Journal of Environmental Quality 25:1216-1220.

Jamieson, R., R. Gordon, D. Joy and H. Lee. 2004. A review of current watershed scale modeling approaches. Agricultural Water Management 70: 1-17.

Jolley, L. W. 2005. The interactions of indicator bacteria and sediments in fresh water streams. Doctoral dissertation. Clemson University, Clemson, SC ,USA.

Kistemann, T., T. Calben, C. Koch, F. Dangendorf, R. Fischeder, J. Gebel, V. Vacata, and M. Exner. 2002. Microbial loading of drinking water reservoir tributaries during extreme rainfall and runoff. Journal of Applied and Environmental Microbiology 68:2188-2197.

LaJeune, Jeffery T., Thomas E. Besser, and D. D. Hancock. 2001. Cattle water troughs as reservoirs of *Escherichia coli*

O157. Journal of Applied and Environmental Microbiology 67: 3053-3057.

McDonald, A., D. Kay, and A. Jenkins. 1982. Generation of fecal and total coliform surges by stream flow manipulation in the absence of normal hydrometerological stimuli. Journal of Applied and Environmental Microbiology 44; 292-300.

Paul, M. J. and J. L. Meyer. 2001. Streams in the urban landscape. Annual Review of Ecological Systems 32: 333-365.

Pettibone, G. W., Kin N. Irvine, and Kelly M. Monahan. 1996. Impact of a ship passage on bacterial levels and suspended sediment characteristics in the Buffalo River, New York. Water Research 30: 2517-2521.

Sherer, B. M., J. R. Miner, J. A. Moore, and J.C. Buckhouse. 1992. Indicator bacterial survival in stream sediments. Journal of Environmental Quality 21:591-595.

Standard Methods for the Examination of Water and Wastewater(2000). 21st edn. American Public Health Association/ American Water Works Association/Water Pollution Control Federation. Washington, D C, USA.

South Carolina Department of Health and Environmental Control. 2004. Watershed Water Quality Assessment: Saluda River Basin. Technical Report No.004-04. Bureau of Water, Columbia, S.C.

Tong, Susanna T. and Wenli Chen. 2002. Modeling the relationship between land use and surface water quality. Journal of Environmental Management 66: 377-393.

USEPA, 1986. Ambient Water Quality Criteria for Bacteria. United States Environmental Protection Agency, Office of Water Regulations and Standards January 1986, Criteria and Standards Division, Washington, DC 20460, EPA440/5-84-002.