# THE PERSISTENCE OF RIVERBED BACTERIAL STORES AND THEIR DISRUPTION BY HUMAN RECREATION

Ryan W. Orear and Nancy Eufemia Dalman

AUTHORS: North Georgia College & State University, Dahlonega, GA 30597.
REFERENCE: *Proceedings of the 2011 Georgia Water Resources Conference*, held April 11–13, 2011, at the University of Georgia.

**Abstract.** Disturbance to riverbed sediments from human recreation allows for *Escherichia coli* (*E. coli*) to become elevated in the water column. To illustrate the role that sediment bacterial stores have in water-borne contamination, three experiments were performed. The first test was a field based bacterial sediment and water comparison designed to confirm that sediments harbor higher bacterial loads than water. Water and sediment samples were collected mid – stream during July, August, and September at five distinct locations. At recreational sites sediment displayed significantly higher *E. coli* levels than water; this trend did not exist at non - recreational sites with minimal sediment disturbance. The second field experiment observed the water – borne concentration of *E. coli* after an induced disturbance to riverbed sediment. A rake was used to disturb a plot of $2m^2$ riverbed and then water samples were collected over a span of 130 seconds, 10 meters downstream of the disturbance. *E. coli* concentrations increased 7.5 - fold and particulates increased 60 - fold in the water column 50-60 seconds after disturbance, then returned to basal levels over the next 70 seconds. The last test was a laboratory based microcosm persistence experiment. Twelve samples received ten grams of sterile sediment plus 100 ml of sterile water. The other twelve samples only received 100 ml of sterile water. All samples were inoculated with $1x10^6$ colony forming units (CFU) of *E. coli. E. coli* persisted on average 5 days longer and at population densities 10 fold higher in water overlaying sediment than in water alone. *E. coli* in all experiments were quantified using the Colilert ® Quanti – tray 2000 system (IDEXX). These results demonstrate that sediments store bacteria for extended periods and as human recreation disturbs sediment, the risk of humans contracting a water-borne infection increases.

## INTRODUCTION

Bacterial counts rise and fall in rivers, streams, and lakes according to the amount of run-off occurring from the surrounding land; this is how rivers initially become contaminated. Some potential sources for contamination are leaking septic tanks, sewage overflow, and run off from animals. This contamination poses a threat for elevated concentrations of microbes in the water. After the water is contaminated, microbes begin to settle in the sediment where they can impact water quality at a later time if and when sediments are disrupted. *Escherichia coli (E. coli)* is a bacterium that is commonly used as an indicator of sewage contamination, and can be easily tested for in the sediment and water. The United States Environmental Protection Agency (EPA) and Georgia Department of Natural Resources, Environmental Protection Division (EPD) mandate bacterial monitoring of recreational waters and have a proposed *E. coli* safe limit of 235 cfu/100ml for a grab sample of water. The presence of *E. coli* can be correlated with pathogenic microbes found in sewage, and thus a public health hazard becomes a concern in developed areas with high levels of water recreation (Whitman and Nevers, 2003).

Previous studies on the Chattahoochee River in Helen, Georgia by the Georgia Mountain Regional Development Center (GMRDC) and Upper Chattahoochee Riverkeeper (UCR) and our lab have reported water – borne *E. coli* levels exceeding the EPA limit, and as recreation increases there is a greater concern of water contamination (GMRDC, 2007; Dalman et. al, 2009). The tubing industry in Helen can see up to 7000 tubers on an average weekend day, and this number is often higher on holiday weekends (personal observation). River visitors tend to have a great deal of contact with the sediment; this may be harmful because riverbed sediments act as a sink for large stores of bacteria (Craig et al., 2004). Bacterial stores are released into the water column through the disruption of sediment (Stephenson and Rychert, 1982). This large-scale disruption of the upper Chattahoochee River's sediment through its commercialized recreation can lead to increased pathogenic bacteria levels in the water column, and anyone who comes in contact with the water risks infection.

The goal of this research is to examine the concentration of *E. coli* in the sediment and overlying water in the Chattahoochee River and to show that *E. coli* levels are consistently higher in riverbeds than in water. The study will also demonstrate that *E. coli* persistent for longer periods of time in sediment as compared to water and that *E. coli* adhering to sediment can be resuspended in the overlying water when sediment experiences a disruption.

## MATERIALS AND METHODS

**Paired sediment and water E. coli comparison.** Water and sediment samples were collected once per month in July, August, and September at seven distinct locations

along the Chattahoochee River; five of the sampling sites were in a stretch of river with high recreational impact and two sites were located in an isolated river section through the Chattahoochee National Forest. Sediment samples were collected by dragging 500 ml sterile Nalgene bottles along the bottom of the river; any water taken in the bottle was decanted off before sealed. Water samples were collected mid-river, at mid-depth, in 100 ml IDEXX sterile bottles (IDEXX Laboratories, Westbrook, ME). All samples were placed on ice until processed in the lab (within 4 hours of collection). Sediment and water *E. coli* analyses were performed using the Colilert® Quanti-tray® 2000 system (IDEXX Laboratories, Westbrook, ME). In the laboratory, ten grams of sediment from each sample were transferred into sterile 100 ml IDEXX bottles along with 100 ml of sterile Chattahoochee River water and Colilert® reagent. Each IDEXX bottle was vortexed for 60 seconds to ensure that bacteria did not adhere to the sediment. The water solution derived from the ten grams of sediment was poured into a sterile IDEXX 97–well Quanti-tray and passed through the IDEXX Quanti-tray sealer, thereby uniformly distributing samples to all wells. The corresponding water samples in 100 ml IDEXX bottles received the Colilert® reagent and were put into trays and sealed in the same manner as the sediment solutions. All trays where then incubated at 35.5$^{o}$ C for 24 hours; wells positive for the presence of *E. coli* fluoresced under U.V. light at 365nm (Mineral UVS-54, Ultraviolet Products, inc., San Gabriel, CA). The number of positive wells for each Quanti-tray was converted into a most-probable number (MPN/100ml) using the supplied IDEXX table. Three 10 gram samples of wet sediment were placed in aluminum envelopes, transferred to a drying oven for 48 hours at 95$^{o}$ C, and reweighed. Dry sediment weight was divided by the MPN to report *E. coli* levels per gram of sediment. Results from all recreational or non–recreational sites were combined for each month and the mean and standard error for each was calculated. Unpaired *t* tests were then conducted to compare mean sediment *E. coli* levels to mean water *E. coli* levels at all recreational or non-recreational sites for each sampling date.

**Sediment disturbance simulation.** This field based experiment was performed in a first order agricultural stream to observe the water–borne concentration of *E. coli* after an induced disturbance to sediment. Stream disturbance was simulated by raking a sediment area of 1m$^2$ at a water depth of 20cm. The sediment was vigorously raked for 30 seconds in order to create a plume of uniform sediment dispersion. The plume was allowed to travel downstream for 5 meters, at which point water samples were collected in IDEXX sterile bottles at 10-second intervals for 130 seconds. Samples were placed on ice and taken to the lab, where turbidity was measured by using a DRT-15CE Turbidmeter (HF Scientific, Inc., Fort Myers, FL). *E. coli* MPN for water samples were measured by using the Colilert® Quanti-tray® 2000 system described above.

**Water – borne E. coli persistence**. *E. coli* survival rates were compared between microcosms containing water alone and microcosms containing water overlaying sediment. Twenty- four Erlenmeyer flasks were separated into two categories: twelve samples received ten grams of sterile sediment plus 100ml of sterile water. The other twelve samples only received 100 ml of sterile water. This experiment was performed in triplicate. A pure culture of *E. coli* (Carolina Biological Supply, Burlington, NC) was inoculated in 10ml of nutrient broth. The *E. coli* cells were collected from the broth culture through centrifugation at 2500g for 10 minutes. The pelleted bacteria were resuspended in 1 ml of phosphate buffer solution (PBS) then washed by centrifugation at 8000g for 10 minutes separating the bacteria from and the phosphate buffer solution once again (Craig *et al.* 2004). The supernatant was removed and the *E. coli* was resuspended in 1.5ml of PBS. Each flask was inoculated with 10μL of the washed solution, giving a starting *E. coli* concentration of 8.2x10$^6$ cells. Microcosms were incubated at 25°C and samples were taken on days 0(one hour after inoculation), 4, 8, 14, 18, 23, 26, 28, 30, 32, 34, and 36. Initially the starting concentrations of *E. coli* were too high to quantify using the IDEXX test, so dilutions of 1000x, 100x, and 10x were taken to quantify the samples when the concentration exceeded the maximum most probable number of the IDEXX test. Once the MPN was calculated for the sample it was multiplied by the dilution factor to obtain the final E. coli count for the microcosm. The decay rate constant was calculated as the slope of the regression line from the two microcosm treatment groups. The decay rate was then used to calculate $t_{90}$ values, which is the time required for a 1-log10 reduction in organism concentration.

## RESULTS

**Bacterial sediment and water comparison**. Figure 1 shows the differences between water and sediment *E. coli* levels at recreational and non – recreational sites in the Chattahoochee River. Sediment *E. coli* counts were consistently higher than water *E. coli* counts at all sites and in all months sampled. Further, the *E. coli* concentrations in the sediments of recreational sites were significantly higher than in the corresponding overlying water for all months tested (June, $p < 0.0001$; August, $p=0.0011$; and September $p=0.0013$). The only significant difference between sediment and water *E. coli* levels at non-recreational sites was in September ($p=0.0230$). All other comparisons between water and sediment at non-recreational sites showed no significant difference between the two groups.

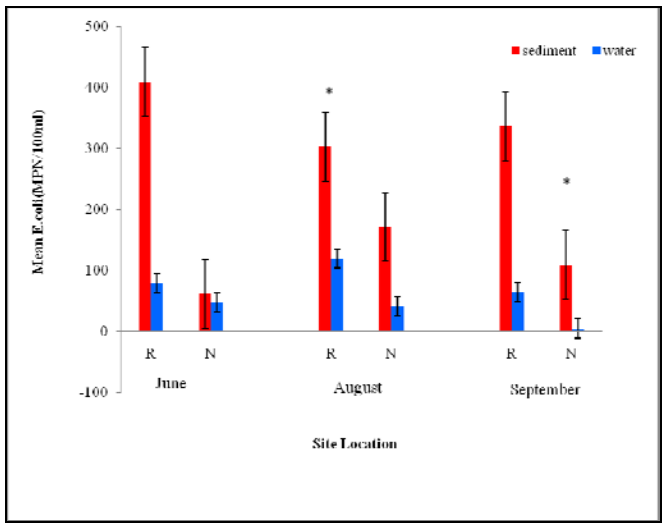


**Figure 1. Sediment and water *E. coli* levels in the Chattahoochee River through Helen, GA. Sediment *E. coli* levels were significantly higher than water *E. coli* levels at recreational sites (R) in June (p<0.0001), August (p=0.0011), and September (p=0.00128). The only significant difference between sediment and water *E.coli* levels at non-recreational sites (N) was in September (p=0.023). * denotes significance at p = 0.05.**

**Sediment disturbance simulation.** A sediment disturbance study was conducted to simulate the type of disruption that might occur in a river with heavy foot travel (figure 2). When sediments were disturbed and suspended by vigorously raking a sandy stream plot of $1m^2$ for thirty seconds, *E. coli* levels in the overlying downstream water peaked at a value that was 7.5 fold higher than in the water prior to sediment disturbance. The increase in water-borne *E. coli* (MPN/100 ml) counts was concomitant with increased suspended solids in the water column, measured as turbidity, which increased by 72 fold. Both *E. coli* counts and turbidity returned to initial levels by 120 seconds after the disturbance.

**Water – borne *E. coli* persistence.** The sediment and water column persistence of *E. coli* was examined in a microcosm experiment. *E. coli* in the presence of water alone showed a consistent decrease in colony number, whereas the *E. coli* in the presence of sediment and water displayed an initial growth period before a steady decline in colony number (figure 3). This is most likely due to the added nutrient availability from the sediment. The decay rate constant was calculated as the slope of the line for *E. coli* count versus time. The decay rate was then used to calculate t90 values, which is the time required for a 1-log10 reduction in organism concentration. The t90 value for the microcosms with water and sediment was 5.70 days and 4.98 days for the microcosm that had only water.

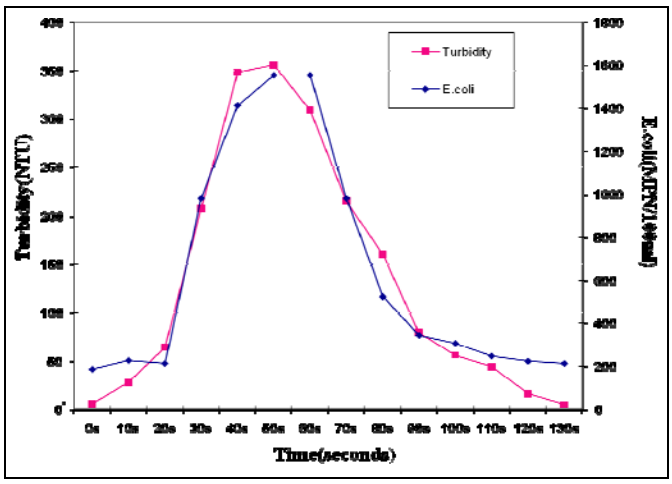


**Figure 2. Suspending solids by vigorously raking sediment for thirty seconds showed elevated *E. coli* levels in the overlying downstream water. The increase in water-borne *E. coli* (MPN/100 ml) counts was concomitant with increased sediment disruption (turbidity).**

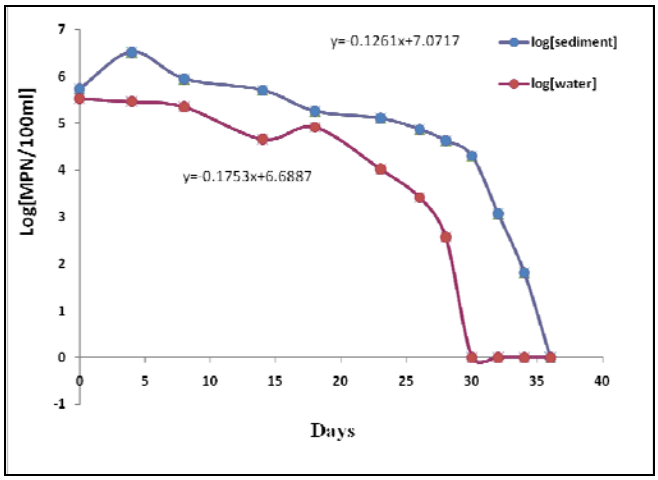


**Figure 3. Mean survival of *E. coli* when incubated at 25° C in sediment and water in a sterile environment for triplicate samples at each time point. The mean decay rate ($t_{90}$) was 5.7 days for *E. coli* exposed to water overlying sediment. *E. coli* incubated in water alone decayed at a rate of 4.9 days indicating that bacterial survival was greater (decay rate was lower) in the presence of sediment than in its absence.**

## DISCUSSION

**River sediments harbor higher *E. coli* counts than overlaying river water.** As microbes settle in the bottom of rivers they are able to adhere to sand, rocks, and other particulate matter where they can live and thrive for long periods of time (Craig et al., 2004). This causes the *E coli* concentration in the sediment to be higher than in the overlying water, except in the event of precipitation where

run-off adds to the bacteria load in the water before it settles into the sediment. Stumpf et. al (2010) examined the input of fecal coliforms during storm flow and base flow, and they discovered that during storm flow the bacteria load on average was 30 to 37 times greater than the base flow bacteria loads. Further, the sediment *E. coli* counts reached 776 CFU/100ml, well above the EPA proposed safe limit of 235CFU/100ml, and acted as a reservoir and source of fecal contamination to overlaying water (Stumpf et. al, 2010). Similar results were seen in our study where sediment *E. coli* counts got as high as 408 CFU/100ml allowing the sediment to be a major source of bacterial contamination during periods of low river flow. When sediment and water *E. coli* levels were compared, it was discovered that on average the bacterial concentration in the sediment was 7.49 fold higher than in the overlying water. These results agree with a study done by Goyal et al (1977) who compared the difference between sediment and water bacterial loads in canals that varied in distance from a sewage drain pipe. Sediments consistently had fecal coliform concentrations that ranged from 1 to 383 times greater than the adjacent water column (Goyal, et. al, 1977).

A significant difference in mean concentration of *E. coli* between sediment and water was found at all recreational sites whereas a significant difference in mean *E. coli* concentration between sediment and water only existed at non-recreational sites in September. Water and sediment *E. coli* levels were always higher at recreational sites as compared to non – recreational sites. The lower *E. coli* counts at non – recreational sites and the lack of significance between sediment and water bacterial load at these sites is probably due to the small amount of point source and non-point source contamination entering the waterway. The non-recreational sites were located in the Chattahoochee National Forest, and were heavily vegetated. It has been shown that vegetation can prevent soil erosion around riparian areas and even trap fecal bacteria from entering waterways (Coyne et. al, 1998).

**The resuspension of solids correlates to elevated *E. coli* concentration.** When sediment is disrupted in a body of water the particulates become suspended in the water column above (Grimes, 1975). This agitation can be caused by added turbulence through rain fall or disturbances from direct contact such as recreation. Sediment disruption can compromise the water quality depending on the chemical and bacterial load of the resuspended debris. In order to evaluate the effect that sediment resuspension had on water quality, a simulated disturbance was conducted. When a sediment plot was raked for 30 seconds, the turbidity, a measure of suspended solids, increased 75 - fold and the elevated turbidity coincided with a 7.5 fold increase in the amount of *E.coli* in the water column. Our findings support those of Stephenson and Rychert (1982) who found that suspending sediment solids increased bacterial load by 10 and 12 fold in rangeland streams. This increase was transient, but showed the effect that just 30 seconds of sediment agitation can have on the overlying water quality. When this effect is multiplied by 7000 tubers walking through river shallows over the course of a weekend day, the disturbance is much more consistent. Visual observation of water samples taken over the course of the summer for another study supported this hypothesis (Jackson et al., 2011). A study done on the Green River reported that the concentration of fecal contaminants increased the most at times of high human recreation on weekends, creating a potential public health hazard (Varness et. al, 2010). On the Chattahoochee River the approved EPA limit of 235 CFU/100ml is exceeded on a regular basis, which can put anyone coming in contact with the water during this time at a high risk of pathogenic infections (Dalman et. al, 2007, Dalman et. al, 2009). Taken together, these results make it evident that bacteria are resuspended in the overlying water when river sediments are disrupted and can have a major effect on water quality.

**Water – borne *E. coli* survive longer when in contact with sediment than when in water alone.** Previous studies have shown that water – borne bacteria in contact with sediment survive longer than bacteria grown only in water (Craig et. al, 2004). To further define the influence of sediment on water – borne bacteria levels, a sterile microcosm study was conducted. *E. coli* grown in the presence of sediment persisted longer than *E. coli* grown in water alone. Craig et. al (2004) showed that bacterial survival in non – sterile microcosms was also consistently greater in water overlying sediment then in just water. The use of sterile microcosms does not completely simulate the natural environment in that they lacked the natural predators, nutrients, light/dark cycles, fluctuating temperature and competition found in their natural environment. However, sterile microcosms allow us to focus solely on the growth and decay of *E. coli* alone. Having established growth curves for *E. coli* under sterile conditions, future studies will compare *E. coli* growth in water alone or water and sediment from the Chattahoochee River using non – sterile microcosms.

The typical die off curve for *E. coli* in aquatic environments involves an initial lag phase then 90 percent mortality within 3 to 5 days (Gerba and Mcleod, 1975). This trend was observed in our study; bacteria exposed to water overlaying sediment displayed 90% average die off in 5.7 days, and bacteria exposed to water alone exhibited 90% mortality in 4.9 days. After inoculation, initial bacterial growth was only seen in the *E. coli* incubated in water and sediment. Although the difference in t90 was 0.8 days, the growth that was displayed by the bacteria exposed to sediment and water was one order of magnitude higher (> 2 million CFU/100ml) than the starting concentration of

bacteria for either of the treatments groups. Burton (1987) suggests that the main reason for the increased survivability in the sediment is due to sorption and sedimentation, which allows for the *E.coli* to be protected from bacteriaphages and parasites that prey on them in aquatic environments. However because our microcosms were sterile environments, the risk of predation was absent. Thus, the growth exhibited by the *E.coli* exposed to sediment was not due to added protection, but likely due to the ability of the microorganisms to utilize sediment associated nutrients (Gerba and Mcleod, 1975).

## CONCLUSIONS

Recreation can disrupt sediment in an aquatic environment considerably (Flack et.al, 1988). Sediment disruption releases bacteria stores, which can have a major effect on the water quality and cause public health concerns to anyone in contact with the water. This may be the case in the Chattahoochee River through Helen, Georgia where on an average weekend day, 777 tubers per hour have been seen walking and floating down the river. This high volume of recreation likely disturbed sediment bacteria stores and was, at least in part, responsible for the elevated concentrations of bacteria in the overlying water. These results support the necessity of GMRDC mandated bacterial monitoring in Helen and suggest that the impacts of human activity and sediment disruption should be accounted for in the development of a monitoring and warning program.

## REFERENCES


Burton,G.A,JR.,D.Gunnison, and Laza,G.R., (1987) Survival of pathogenic bacteria in various freshwater sediments. Applied and Environmental Microbiology.53, 633-638.

Craig,D.L.,H.J. Fallowfield, and N.J. Cromar, (2004) Use of microcosms to determine persistance of *Escheri chia coli* in recreational coastal water and sediment validation with in situ measurements. Journal of Applied Microbiology. 96,922-930.

Coyne, M.S.,Gilfillen, R.A., Villalba, A., Zhang, Z., Rhodes, R.,Dunn,.L, and Blevins, R.L., (1998) Fecal bacteria trapping by grass filter strips during simulated rain. Journal of Soil and Water Conservation. 53,140-145.

Dalman, N.E., Smith, A.M., Pschandl, C. M. & Van Cleave, R.M. (2007). Effect of human recreation on *Escherichia coli* levels in the Chattahoochee River in Helen, GA. In K.J. Hatcher (ed.) Proc. 2007 GWRC Conference, March 27 – 29, 2007, University of Georgia, Athens, GA.

Dalman, N. E., Barrett, L. B., & Peck, B. D. (2009). Impact of human activity on *Escherichia coli* levels in a recreational river. Journal of Environmental Detection 2(1), 17-40.

Donovan, E.P., Staskal,D.F., Unice,K.M., Roberts, J.D., Haws, L.C, Finley, B.L., and Harris, M.A, (2008) Risk of gastrointestinal disease associated with exposure to pathogens in the sediment of the Lower Passaic River. Applied and Environmental Microbiology. 74, 1004-1018.

Flack, J.E, Medine, A.J., and Hansen-Bristrow,K.J., (1988) Stream quality in a mountain recreation area. Mountain Research and Development, 8,11-22.

Gerba,C.P., and J.S McLeod, (1976) Effects of sediments on the survival of *Escherichia coli* in marine waters. Applied and Environmental Microbiology. 32,114-120.

GMRDC (2007). Regional agenda 2007 annual update .Available at http://www.grmdc.org/index_regional_agenda.pdf. Georgia Mountain Regional Development Center, Gainesville, Georgia.

Grimes, D.J., (1975) Release of sediment-bound coli forms by dredging. Applied and Environmental Microbiology. 109-111

Goyal S.M., Gerba,C.P, and Melnick, J.L., (1977) Occurrence and distribution of bacterial indicators and pathogens in canal communities along the Texas coast. Applied and Environmental Microbiology. 34, 139-149.

Jackson, T.E., McMullan, G.,Orear R.W, Smith A.C., Fuller,R.C., and Dalman, N.E., (2011) A comparison of *Escherichia coli* levels in the Chattahoochee River in Helen, Georgia between drought and non-drought years, Proc. 2011 GWRC Conference, in review.

Marino, R.P, and Gannon, J.J., (1991) Survival of fecal coliforms and fecal *Streptococci* in storm drain sediment. Water Resource.25, 1089-1098.

Savageau, M.A., (1983) *Escherichia coli* habitats, cell types, and molecular mechanisms of gene control. American Naturalist. 122, 732-744 .

Stephenson, G.R., and Rychert, R.C., (1982) Bottom sediment: A reservoir of *Escherichia coli* in rangeland streams. Journal of Rangeland Mangement. 35,119-123.

Stumpf, C.H., Piehler, M.F., Thompson, S., and Noble ,R.T., (2010) Loading of fecal indicator bacteria in North Carolina tidal creek headwaters: hydrographic patterns and terrestrial runoff relationships. Water resource.16, 4704-15 .

Whitman, R.L, and Nevers, M.B, (2003) Foreshore sand as a source of *Escherichia coli* in nearshore water of a Lake Michigan beach. Applied and Environmental Microbiology. 69, 5555-5562.

Varness, K.J, Pacha, R.E, and Lapen, R.F., (1978) Effects of dispersed recreational activities on microbiological quality of forest surface water. Applied and Environmental Microbiology.36, 95-104.